The Role of Endoscopy, Biomarkers and Imagiology in the Clinical Management of Inflammatory Bowel Disease Patients

Contributo da Endoscopia, Biomarcadores e Imagiologia na evolução clinica dos doentes com Doença Inflamatória Intestinal

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Abbreviations

Anti- TNFα *Anti-tumor necrosis factor alfa*

BD *Bowel damage*

CD *Crohn´s Disease*

CDAI *Crohn´s Disease Activity Index*

CECDAI *Capsule Endoscopy Crohn´s Disease Activity Index*

CMV *Cytomegalovirus*

CTE *Computed Tomography Enterography*

CU Colite Ulcerosa

DAE *Device Assisted Enteroscopy*

DAMP *Damage-Associated Molecular Pattern*

DC Doença de Crohn

DEB Dilatação Endoscópica com Balão

DII Doença Inflamatória Intestinal

DNA *Deoxyribonucleic acid*

EBD *Endoscopic Balloon Dilation*

EBV *Epstein-Barr virus*

ECCO *European Crohn´s and Colitis Organization*

ESGE *European Society of Gastrointestinal Endoscopy*

FC *Fecal calprotectin*

FL *Fecal lactoferrin*

HSSV *Human Herpes simplex virus*

IBD *Inflammatory Bowel Disease*

MaRIA *Magnetic Resonance Index of Activity*

MH *Mucosal Healing*

MNV *Murine norovirus*

MRE *Magnetic Resonance Enterography*

mSES-CD *modified Simple Endoscopic Score for Crohn's disease*

NK-cells *Natural killer cells*

PCR *Polymerase Chain Reaction*

PICE *Pan-Intestinal Capsule Endoscopy*

PTLD *Post-Transplant Lymphoproliferative disease*

SB *Small Bowel*

SES-CD *Simple Edoscopic Score for Crohn´s Disease*

SICUS *Small Intestine Contrast Ultrasonography*

STRIDE *Selecting Therapeutics Targets in Inflammatory Bowel Disease*

TAC Tomografia Axial Computorizada

UCEIS *Ulcerative Colitis Endoscopic Index of Severity*

UC *Ulcerative Colitis*

VCE *Video Capsule Endoscopy*

List of Publications

Along with the specific studies designed and conducted for the present Thesis, the author was also actively involved in several other projects that were related with this thesis, and which will also be presented here.

The list of publications conducted for this thesis is hereby presented:

1. **Lopes S**, Andrade P, Conde S, Liberal R, Dias CC, Fernandes S, Pinheiro J, S. Simões J, Carneiro F, Magro F, Macedo G. **Looking into Enteric Virome in Patients With IBD: Defining Guilty or Innocence?** Inflamm Bowel Dis 2017;Aug 23 (8):1278-1284.

2. **Lopes S**, Andrade P, Afonso J, Rodrigues-Pinto E, Dias CC, Macedo G, Magro F. **Correlation Between Calprotectin and Modified Rutgeerts Score.** Inflamm Bowel Dis 2016; 22:2173-2181.

3. **Lopes S**, Andrade P, Rodrigues-Pinto E, Afonso J, Macedo G, Magro F. **Fecal Markers Levels as Predictors of the Need for Endoscopic Balloon Dilation in Crohn´S Disease Patients with Anastomotic Strictures.** World J Gastroenterol 2017 September 21; 23 (35): 6482-6490.

4. **Lopes S**, Rodrigues-Pinto E, Andrade P, Afonso J, Baron TH, Magro F, Macedo G. **Endoscopic Balloon Dilation of Crohn's Disease Strictures – Safety, Efficacy and Clinical Impact.** World J Gastroenterol 2017 November 7; 23 (41): 7397-7406.

5. **Lopes S**, Andrade P, Afonso J, Cunha R, Rodrigues-Pinto E, Ramos I, Macedo G, Magro F. **Monitoring Crohn´s Disease Activity: Endoscopy, Fecal Markers and CT Enterography.** In press in Therap Adv Gastroenterol 2018.

6. **Lopes S**, Andrade P, Cunha R, Magro F. **Transmural Healing in Crohn´s Disease: Beyond Mural Findings.** Dig Liver Dis 2018 Jan; 50 (1): 103-104.

7. Santos-Antunes J, Cardoso H, **Lopes S**, Marques M, Nunes ACR, Macedo G. **Capsule Enteroscopy is Useful for the Therapeutic Management of Crohn´s Disease.** World J Gastroenterol 2015 November 28; 21 (44): 12660-12666.

The other projects that resulted in related publications are:

1. Magro F, Lopes J, Borralho P, **Lopes S**, Coelho R, Cotter J, Dias de Castro F, de Sousa HT, Salgado M, Andrade P, Vieira AI, Figueiredo P, Caldeira P, Sousa A, Duarte MA, Avila F, Silva J, Moleiro J, Mendes S, Giestas S, Ministro P, Sousa P, Gonçalves AR, Gonçalves B, Oliveira A, Rosa I, Rodrigues M, Chagas C, Dias CC, Afonso J, Geboes K, Carneiro F; Portuguese IBD Study Group (GEDII).**Comparison of different histological indexes in the assessment of ulcerative colitis activity and their accuracy regarding endoscopic outcomes and faecal calprotectin levels.** Accepted for publication in Gut 2018.

2. Magro F, **Lopes S**, Coelho R, Cotter J, Dias de Castro F, Tavares de Sousa H, Salgado M, Andrade P, Vieira AI, Figueiredo P, Caldeira P, Sousa A, Duarte MA, Ávila F, Silva J, Moleiro J, Mendes S, Giestas S, Ministro P, Sousa P, Gonçalves R, Gonçalves B, Oliveira A, Chagas C, Torres J, Dias CC, Lopes J, Borralho P, Afonso J, Geboes K, Carneiro F; Portuguese IBD Study Group [GEDII]. **Accuracy of Faecal Calprotectin and Neutrophil Gelatinase B-associated Lipocalin in Evaluating Subclinical Inflammation in UlceRaTIVE Colitis - the ACERTIVE study.** J Crohns Colitis. 2017 Apr 1;11(4):435-444.

3. Magro F, Dias CC, Coelho R, Santos PM, Fernandes S, Caetano C, Rodrigues Â, Portela F, Oliveira A, Ministro P, Cancela E, Vieira AI, Barosa R, Cotter J, Carvalho P, Cremers I, Trabulo D, Caldeira P, Antunes A, Rosa I, Moleiro J, Peixe P, Herculano R, Gonçalves R, Gonçalves B, Tavares Sousa H, Contente L, Morna H, **Lopes S**. **Impact of Early Surgery and Immunosuppression on Crohn's Disease Disabling Outcomes.**

4. Magro F, Lopes SI, Lopes J, Portela F, Cotter J, **Lopes S**, Moreira MJ, Lago P, Peixe P, Albuquerque A, Rodrigues S, Silva MR, Monteiro P, Lopes C, Monteiro L, Macedo G, Veloso L, Camila C, Afonso J, Geboes K, Carneiro F; Portuguese IBD group [GEDII]. **Histological Outcomes and Predictive Value of Faecal Markers in Moderately to Severely Active Ulcerative Colitis Patients Receiving Infliximab.** J Crohns Colitis. 2016 Dec;10(12):1407-1416.

5. Rodrigues-Pinto E, Cardoso H, Rosa B, Santos-Antunes J, Rodrigues S, Marques M, **Lopes S**, Albuquerque A, Carvalho P, Moreira M, Cotter J, Macedo G. **Development of a predictive model of Crohn's disease proximal small bowel involvement in capsule endoscopy evaluation.** Endosc Int Open. 2016 Jun;4(6):E631-6.

6. Albuquerque A, Cardoso H, Marques M, Rodrigues S, Vilas-Boas F, **Lopes S**, Dias CC, Macedo G. **Predictive factors of small bowel patency in Crohn's disease patients.** Rev Esp Enferm Dig. 2016 Feb;108(2):65-70.

7. Magro F, Santos-Antunes J, Albuquerque A, Vilas-Boas F, Macedo GN, Nazareth N, **Lopes S**, Sobrinho-Simões J, Teixeira S, Dias CC, Cabral J, Sarmento A, Macedo G. **Epstein-Barr virus in inflammatory bowel disease-correlation with different therapeutic regimens.** Inflamm Bowel Dis. 2013 Jul;19(8):1710-6.

8. Rodrigues S, Pereira P, Magro F, **Lopes S**, Albuquerque Aw, Lopes J, Carneiro F, Macedo G. **Dysplasia surveillance in an ulcerative colitis patient: successful detection with narrow band imaging and magnification.** J Crohns Colitis. 2011 Feb;5(1):54-6.

Outline of Thesis

In Chapter I, a general introduction and rational concerning the subject chosen for this Thesis is presented. This includes the available evidence of the role of different diagnostic and therapeutic methods in the evaluation of disease activity and resolution of complications, and the evidence demonstrating the importance of infectious agents in the pathogenesis and disease activity in IBD.

In Chapter II, the aims of each of the six studies conducted for the present Thesis are presented.

In Chapter III, the publications that build the core for the present Thesis are presented.

In Chapter IV, an integrated discussion of all the articles is presented supporting the major conclusions of these Thesis.

In Chapter V, the conclusions are presented and areas for clinical research are pointed out.

Abstract

Inflammatory bowel disease (IBD) is a chronic, relapsing, inflammatory condition of the gastrointestinal tract, encompassing two diseases: Crohn´s disease (CD) and Ulcerative colitis (UC). The evidence that incidence and prevalence of IBD correlates with industrialization and westernalization of populations has driven much attention to possible environmental triggers of disease. IBD is believed to manifest in a genetically predisposed individual who reacts inappropriately to the gut microbiome after exposure to an external trigger. In this context much attention has been driven recently by human virome. Some studies have proved an interaction between eukaryotic viruses and IBD risk genes, suggesting a role for enteric virome in IBD pathogenesis. It has also been demonstrated that the viral population may be influenced by immunosuppressive therapy, with some specific viruses taking advantage of the immunosuppressive status of the host. Despite the amount of evidence trying to implicate viral dysbiosis in the pathogenesis of IBD it has been difficult to confirm results across validation cohorts. The Human Herpesviridae (HHV) family is a DNA virus family encompassing Cytomegalovirus (CMV), Epstein Barr Virus (EBV) and Human Herpes simplex virus 6 (HSSV-6), that have in common being highly prevalent in adulthood (>95%) and inducing lifelong latency in the host. During periods of immunosuppression they may reactivate and have a role in exacerbating chronic inflammatory diseases. Data from the post-transplant setting demonstrate virus reactivation with clinical implications in this state of intense immunosuppression suggesting that this effect might also happens in IBD patients. Until the present time, it remains to be proved if the changes observed in number and richness of virus in IBD are biologically and clinical relevant in terms of disease phenotype, behavior and response to therapy.

The access to the intestinal mucosa, healthy or involved in the disease process, is achieved with different modalities of endoscopy which allow the direct observation and histological appraisal of the inflammatory features within the gut. Besides its contribution to the clarification of disease pathogenesis, endoscopy has also an important role in disease monitoring and evaluation of response to therapy.

In the last decades, the way we approach and manage IBD has evolved enormously. The concepts of mucosal healing, transmural healing and bowel damage have changed the goals of therapy and subsequent follow-up. At the present time, the standard of care implies a close monitoring of the inflammatory burden of the disease in order to avoid disability. As clinical symptoms and serum biomarkers have proved to be inaccurate in predicting disease activity, and because endoscopy implies a certain degree of invasiveness, other noninvasive methods have been investigated. Fecal markers, cross-sectional imaging modalities and video capsule endoscopy (VCE) have gained interest in disease monitoring. Despite all the encouraging data about their accuracy in disease evaluation, colonoscopy remains the gold standard against which all other methods are compared and validated. In addition to the direct evaluation of lesions severity and extension, endoscopy allows the performance of therapeutic techniques, like endoscopic balloon dilation (EBD). This technique not only contributes to symptom resolution and consequent bowel preservation but also allows to diagnose recurrence in the post-operative setting.

With this Thesis we sought to study how some endoscopic modalities, fecal markers and imagiology could contribute to the management of IBD patients in specific clinical settings.

First, we investigated the prevalence and role of some members of the Human Herpesviridae family in disease pathogenesis and course. We studied and performed endoscopy in a group of 95 IBD patients (UC and CD patients) and 50 healthy subjects (HC). EBV and CMV were more prevalent in the IBD population, and in areas of mucosa with endoscopic activity. The prevalence of HHV6 was similar between patients and HC. CMV median viral load was higher in diseased mucosa of UC patients while EBV median viral load was higher in inflamed mucosa of CD patients. We did not find any influence of the immunosuppressive treatment regimens in viral serum prevalence.

The second aim of this thesis was to assess the accuracy of fecal markers in predicting disease recurrence after surgery, comparing two endoscopic scores- the Rutgeerts score and the Modified Rutgeerts score. In this study of 99 patients, FC and FL levels proved to be higher

in patients with endoscopic recurrence compared to endoscopic remission. The Modified Rutgeerts score performed better than the Rutgeerts score with higher sensitivity, negative predictive value and accuracy in predicting recurrence. Cut-off values for both markers were established.

The third and fourth objectives were to evaluate the accuracy of fecal markers in predicting recurrence in patients with asymptomatic anastomotic strictures, selecting patients to EBD, and to evaluate the efficacy and safety of this technique. In our group of 178 patients who underwent colonoscopy, 48 were successfully dilated and recurrence was diagnosed only after dilation in 22 patients. Fecal markers were good predictors of endoscopic recurrence and can be used as guidance to EBD. EDB demonstrated to be an effective and safe alternative to surgery, with the possibility of being repeated as needed.

In order to monitor response to therapy we evaluated the performance of computed tomography enterography (CTE) and fecal markers compared to endoscopy at diagnosis and in the first year after beginning therapy. In a group of 29 consecutive patients with newly diagnosed CD we performed endoscopy, CTE and fecal calprotectin (FC) at M0 and M12. We found a good correlation between the 3 methods at both time points. In patients with endoscopic remission at one year, CTE findings of inflammatory activity significantly improved, CTE score decreased and FC values normalized. A combination of both noninvasive markers may be used to monitor response to therapy, with the advantage of evaluating transmural healing.

The last aim of this project was to evaluate the impact of VCE in CD management, since its role in treatment guidance is not completely defined. In a group of 83 patients with long-term disease in clinical remission, VCE identified unknown upper tract involvement in 49 patients, and its findings translated in treatment changes in 40% of patients. These results highlight the importance of small bowel mucosal imaging in the management of CD.

In conclusion, despite the promising results obtained with noninvasive methods in disease monitoring, being easy to repeat and gaining patient general acceptance, their definitive place in patients' evaluation

still needs to be defined. Direct mucosal observation, either by colonoscopy or VCE, still has a major role in IBD management. Cross sectional imaging modalities and fecal markers may and should be incorporated into patients' algorithm management, selecting patients to endoscopy. Validated scores are needed in CTE, with special attention to mesenteric findings of inflammation, and in endoscopy, dissemination of the Modified Rutgeerts score should be implemented. EBD should be performed not only to induce symptoms resolution in stenotic patients but also to gain access to otherwise inaccessible bowel segments, allowing therapeutic adjustments as needed.

Resumo

A Doença Inflamatória Intestinal (DII) é uma doença crónica, inflamatória e recidivante do tubo digestivo, englobando a Doença de Crohn (DC) e a Colite Ulcerosa (CU). A associação entre o aumento da sua incidência e prevalência e a industrialização e ocidentalização das populações tem despertado muito interesse para um potencial papel de agentes ambientais na sua etiopatogénese. Assume-se que a DII se desenvolve num indivíduo geneticamente predisposto que reage de forma inadequada ao seu microbioma após exposição a um estímulo externo. Neste contexto, nos últimos anos o viroma humano tem despertado muita atenção da comunidade científica. Alguns estudos demonstraram uma interação entre vírus eucarióticos e genes de susceptibilidade para a DII, sugerindo um papel para o viroma na patogénese da DII. O reconhecimento de que a terapêutica imunossupressora influencia a população vírica entérica, coloca a hipótese de alguns vírus específicos poderem aproveitar o estado imunossuprimido do hospedeiro para promoverem o desenvolvimento de doença. Apesar de evidência cientifica implicando a disbiose viral na patogénese da DII, ainda não foi possível confirmar estes resultados em coortes de validação. Os vírus da família Herpesviridae humana são vírus de ADN englobando o CMV, EBV e HSSV- 6. Estes vírus têm em comum a elevada prevalência na idade adulta (> 95%) e persistirem num estado de latência ao longo da vida do hospedeiro. Durante períodos de imunossupressão a sua reativação pode ocorrer, com consequente exacerbação de doenças crónicas. Os dados existentes da transplantação demonstram que em estados de imunossupressão intensa se verifica a reativação vírica com implicações clínicas, sugerindo que estes efeitos também possam ocorrer em doentes com DII. Até ao momento, continua por inequivocamente demonstrado, se as alterações de número e variabilidade de vírus na DII são biológica e clinicamente relevantes em termos de fenótipo, comportamento e resposta da doença ao tratamento.

As várias modalidades endoscópicas disponíveis garantem o acesso á mucosa doente e saudável, e permitem a observação directa e histológica das manifestações inflamatórias no tubo digestivo. A endoscopia para além de contribuir para o esclarecimento da patogénese da

doença tem também um papel na sua monitorização e na avaliação da resposta ao tratamento.

Os conceitos de cicatrização da mucosa, cicatrização transmural e dano intestinal ("*bowel damage*"), introduzidos recentemente na abordagem da DII, modificaram os objetivos terapêuticos. Na actualidade o seguimento ideal destes doentes implica a monitorização regular da carga inflamatória de forma a evitar a irreversibilidade das lesões. Como os sintomas e os biomarcadores séricos demonstraram ser imprecisos na avaliação da atividade da doença, e pela invasividade que a endoscopia representa, foi explorada a utilização de outros métodos não-invasivos alternativos. Os marcadores fecais, os métodos de imagem radiológicos e a vídeo cápsula endoscópica (VCE) têm vindo a ganhar interesse na monitorização da doença.

Apesar dos resultados encorajadores da acuidade destes métodos na avaliação da actividade da doença, a colonoscopia continua a ser a referência para comparação e validação dos restantes métodos. A endoscopia, além da determinação da gravidade e extensão das lesões, permite a realização de técnicas terapêuticas, como a dilatação endoscópica com balão (DEB). Esta técnica para além da resolução sintomática, permitindo a preservação do segmento intestinal, possibilita o diagnóstico da recorrência da doença no pós-operatório.

Com esta Tese, procuramos estudar como alguns métodos endoscópicos, os marcadores fecais e a imagiologia podem contribuir para o acompanhamento clínico dos doentes com DII em contextos clínicos específicos.

No primeiro estudo, investigamos a prevalência e o papel de alguns membros da família Herpesviridae na patogénese e na evolução da doença. Realizamos colonoscopia a 95 doentes com DII (DC e CU) e 50 indivíduos saudáveis (HC). Os vírus EBV e CMV foram mais prevalentes na população com DII e em áreas de mucosa com atividade endoscópica. A prevalência do HHV6 foi semelhante entre doentes e HC. A carga vírica média do CMV foi mais elevada na mucosa ulcerada dos doentes com

CU, enquanto a carga vírica média do EBV foi maior na mucosa ulcerada dos doentes com DC. Não encontramos qualquer correlação entres os regimes terapêuticos imunossupressores e a prevalência vírica sérica.

O segundo objetivo desta tese foi avaliar a acuidade dos marcadores fecais como predictores de recorrência após a cirurgia e comparar dois scores endoscópicos - o *score* de Rutgeerts e o *score* de Rutgeerts modificado. Neste estudo com 99 doentes, os níveis de calprotectina fecal e lactoferrina fecal demonstraram ser mais elevados em doentes com recorrência endoscópica em comparação com doentes em remissão endoscópica. O *score* de Rutgeerts modificado demonstrou ser superior ao *score* de Rutgeerts na predição de recorrência, com maior sensibilidade, valor preditivo negativo e acuidade. Foram estabelecidos valores de *cut-off* para os dois marcadores fecais.

O terceiro e o quarto objetivos foram avaliar a acuidade dos marcadores fecais na predição de recorrência em doentes com estenose assintomática da anastomose ileocólica selecionando os doentes para DEB, e avaliar a eficácia e segurança desta técnica. No nosso grupo de 178 doentes submetidos a colonoscopia, 48 foram dilatados com sucesso e foi feito o diagnóstico de recorrência apenas após a dilatação em 22 pacientes. Os marcadores fecais revelaram ser preditores de recorrência endoscópica podendo ser utilizados como indicadores da necessidade de DEB. A DEB demonstrou ser uma alternativa eficaz e segura à cirurgia, com a possibilidade de poder ser repetida se necessário.

A fim de monitorizar a resposta à terapêutica, avaliamos o desempenho da enterografia por tomografia axial computorizada (TAC) e dos marcadores fecais em comparação com a endoscopia, á data do diagnóstico e no primeiro ano após o início de tratamento. Num grupo de 29 doentes consecutivos com DC recém-diagnosticada, foi realizada endoscopia, enterografia por TAC e doseamento de calprotectina fecal ao mês 0 e ao mês 12. Os resultados demonstraram existir uma boa correlação entre os 3 métodos ao diagnóstico e ao primeiro ano de seguimento após o início do tratamento. Nos doentes em remissão endoscópica ao ano, os sinais de atividade inflamatória na enterografia

por TAC melhoraram significativamente, o score da enterografia por TAC diminuiu e os valores de calprotectina fecal normalizaram. A combinação dos dois marcadores não invasivos poderá ser utilizada para monitorizar a resposta á terapêutica, com a vantagem de avaliar a cicatrização transmural.

O último objetivo deste projeto foi avaliar o impacto da VCE no seguimento da DC, uma vez que o seu papel no estabelecimento da estratégia terapêutica não está totalmente definido. Em 83 doentes com doença de longa duração em remissão clínica, a VCE diagnosticou o envolvimento do trato digestivo superior em 49 doentes, condicionando alterações terapêuticas em 40% dos doentes. Estes resultados salientam a importância da observação da mucosa do intestino delgado na abordagem da DC.

Em conclusão, apesar dos resultados promissores dos métodos não invasivos na abordagem da DII, com facilidade de repetição e maior aceitação pelos doentes, o seu posicionamento no algoritmo de avaliação precisa ser definido. A observação direta da mucosa, seja por colonoscopia ou VCE, ainda possui um papel fundamental na abordagem da DII. Os métodos de imagem radiológicos e os marcadores fecais poderão e deverão ser incorporados no algoritmo de seguimento, selecionando os doentes para endoscopia. É necessário o desenvolvimento e validação de *scores* na enterografia por TAC, incorporando os achados mesentéricos de inflamação. Na endoscopia, a disseminação do *score* de Rutgeerts modificado deve ser fomentada. A DEB deve ser realizada não só para resolução sintomática em doentes com estenoses, mas também para permitir a avaliação de segmentos intestinais de outra forma inacessíveis, possibilitando modificações terapêuticas se necessário.

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Rational
And Introduction

Inflammatory bowel disease (IBD) is a chronic, relapsing, inflammatory condition of the gastrointestinal tract, encompassing two different diseases: Crohn´s disease (CD) and Ulcerative colitis (UC). CD was described for the first time in 1932 at the American Medical Association Meeting. In this session, a paper by Crohn, Ginzburg and Oppenheimer was presented, entitled "*Regional Enteritis, a Pathological and Clinical Entity*"[1]. A disease description was made, based on a study of 14 cases up to 1932. The first report of UC goes back to the second half of nineteen century when Samuel Wilks reported a case of a young woman who died of severe bloody diarrhea. Initially reported as sporadic diseases, since the middle of the 20th century, the incidence of both diseases has increased in the western world. Currently it is estimated that the prevalence of IBD in western countries is up to 0,5% of the general population. Defined as a chronic, incurable disease with a low mortality rate diagnosed predominantly at a young age, the pool of newly diagnosed patients every year exponentially increases the number of prevalent cases.

Since its first report, much as evolved in terms of diagnosis and treatment in IBD.

The evidence that incidence and prevalence of IBD correlates with industrialization and westernalization of populations has driven much attention to the environmental triggers of disease. IBD is believed to manifest in a genetically predisposed individual who reacts inappropriately to the gut microbiome after exposure to an external trigger. A great deal of investment and research has focused on how immune dysfunction induces and maintains a chronic inflammatory state in IBD, resulting in an improved understanding of its immunopathogenesis. Despite that, a full comprehension of the cause and mechanisms of IBD is still lacking. The immune pathogenic mechanisms are only one of the arms of the equation, interacting with genetic, microbiome and environmental factors.

At the present time, recognition of immunological mediators of the disease has led to the development of more directed therapies but data are still missing in terms of unravel the causal agent(s). It is widely accepted that no single component of this equation can alone trigger

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the disease and a combined disruption of all the elements controlling intestinal homeostasis is necessary to disease initiation and mediation.

The follow-up of IBD patients is challenging, since it implies an individualized strategy, which needs to be adjusted to the disease and the patient. In the last decades, the way we approach and manage this condition has evolved tremendously. The development of new and more powerful drugs, capable of reverting structural damage, the increasing use of more accurate biological markers of inflammation to early detect and predict disease activity and the availability of more accurate imaging technics, have changed the goal of the follow-up. Actually, the standard of care implies a close monitoring of the inflammatory burden of the disease in order to avoid disability. This includes taking into account different parameters such as symptoms, biological markers, endoscopy and other imaging modalities, and adjust therapy according.

Metabolomics

The increasing progress in recent years in metabolomics as shifted again the attention to disease pathogenesis, mainly to the importance of the microbiome. There are some pathological features of CD suggestive of an infectious etiology, such as aphthous ulcers of the mucosa, mural abscesses, suppurative fistulas, and macrophage and epithelioid cell granulomas. The possible implication of an infectious agent in CD was first postulated by Dalziel in 1913, before the classic description of CD, when he noted the similarities with Johne's disease (a diarrheal disease in animals, caused by Mycobacterium avium paratuberculosis, with epithelial granulomas). However, no infectious agent has yet been unequivocally identified as inducer of CD.

It has been well demonstrated that the composition of the luminal and mucosal bacteria differs in patients with IBD compared to the general population, with a decreased prevalence of anti-inflammatory bacteria. The increased incidence of IBD worldwide and especially in countries with previous low incidences, and the increased risk for developing IBD among people who migrate from low to high incidence zones appear to support the notion of environment-driven epigenetic modifications. It is known that geography and ethnicity, as well as diet, antibiotic consumption early in life, and certain lifestyle (like smoking and oral contraceptive use) influences microbiota composition. This may explain the recent changes in the incidence of microbiota-related disorders, in which IBD is included. The identification of several gene loci associated with the development of IBD has highlighted the existence of a gene-microbe-environment interaction in IBD pathogenesis. Key IBD risk genes include genes involved in the pathway of sensing and response to microbiota. Whether tissue damage results from an abnormal immune response to a normal microbiota or from a normal immune response to an abnormal microbiota remains to be definitely and unequivocally answered. Possibly more important than the type and quantity of bacteria present in the gut and altered in IBD, is the functional consequence of that alteration, interfering with pathways involved in inflammation regulation.

Much less investigated has been the role of virome in IBD. In fact, virus comprise the most abundant biological entities within the gut, greatly outnumbering bacteria. It is known that the viral component of the microbiome has the potential to influence host physiology and homeostasis. Enteric virome consists of bacteriophages and eukaryotic viruses[2-7]. Like the bacterial microbiome, the human gut virome is characterized by significative changes during the first 2 years of life. In adult life bacteriophages decrease in richness and diversity, and their composition shifts significantly; these changes are associated with increased richness of eukaryotic viruses, which is believed to depend strictly on environmental influences[8].

Animal studies have proved an interaction between eukaryotic viruses and IBD risk genes, indicating that members of the virome may contribute to IBD[9-12]. In a study of germ-free or antibiotic treated mice infected with murine norovirus (MNV), Kernbauer *et al*[13] demonstrated that the beneficial function of commensal bacteria in the gut may be replaced by virus. This study supports the hypothesis that similarly to bacteria, eukaryotic viruses have the capacity to support intestinal homeostasis and shape mucosal immunity. Most of the research on the potential role of virus in IBD has focused on bacteriohages due to their influence on bacterial populations. The interaction of persistent virus infection with commensal microbiome and immune system is believed to be responsible for a particular immunophenotype. Virus-susceptibility gene interaction could explain the clinical heterogeneity and the great variability of response to specific treatments options between different patients.

The role of gut virome is IBD pathogenesis is just beginning to be understood, but there is evidence of being altered in patients with IBD with specific changes assessed between UC and CD. Normal *et al* demonstrated that patients with IBD have an increased number and richness of phage virus, with specific populations identified in each disease type, associated with bacterial dysbiosis[14]. It has also been demonstrated that viral population may be influenced by immunosuppressive therapy, with some specific viruses taking advantage of the immunosuppressive status of the host[15-18].

Despite the amount of evidence trying to implicate bacterial and viral dysbiosis in the pathogenesis of IBD it has been difficult to confirm

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[3] Finkbeiner SR *et al.* Virology Journal. 2008;5:117.

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[8] Lim ES *et al.* Nature Medicine. 2015.

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[15] Perez-Brocal V *et al.* Inflammatory Bowel Diseases. 2015.

[16] Madsen CD *et al.* HIV Clinical Trials. 2002.

[17] Thom K *et al.* Journal of Medical Virology. 2007.

[18] McElvania TeKippe E *et al.* PloS One. 2012.

results across validation cohorts. Other potential interest of virus in IBD would be their role as biomarkers of disease, as several works have demonstrated an increased richness and biodiversity of phage population compared to controls[14,19,20].

It has been shown that latent viral infections, like herpesvirus infections, possess a role in exacerbating chronic inflammatory diseases[21-24]. The Human Herpesviridae family is a DNA virus family encompassing cytomegalovirus (CMV), Epstein-Barr virus (EBV) and Human Herpes simplex virus (HSSV)-6. They have in common being highly prevalent in adulthood (>95%) and inducing lifelong latency in the host, with reactivation during periods of immunosuppression. There is a large number of studies trying to implicate CMV and EBV in IBD pathogenesis and/or disease course. Although CMV colitis in immunocompetent patients is extremely rare, its association with IBD has been reported for more than half a century[25]. The exact prevalence of CMV in IBD population is not entirely known. Several studies have shown a higher prevalence of CMV in IBD patients, that have not been replicated by others. This may be explained by selection bias and the methods used to diagnose the infection. While latent or subclinical infection is diagnosed by the presence of a positive CMV IgG, the diagnosis of CMV colitis should not be based on the presence of CMV IgM, but rather on viremia detection and presence of the virus in the colon.

There are 3 diagnostic methods to detect CMV in colonic mucosa: haematoxylin and eosin staining, immunohistochemistry and polymerase chain reaction (PCR). PCR is the most sensitive and specific of the 3 methods in diagnosing CMV disease, being able to determine viral load. Qualitative PCR can detect the presence of CMV DNA in colonic mucosa, but that does not discriminate between infection and disease. On the other hand, the quantitative method seems more attractive, allowing for the quantification of viral DNA. That has proved to be useful on the context of EBV and CMV infection in the post-transplant population. Nevertheless, in IBD no cut-off has until now been defined, although some papers state that a CMV viral load > 250 copies/mg of tissue in patients with active UC is predictive of nonresponse to steroids, infliximab and cyclosporine[26].

[19] Wagner J *et al.* Inflammatory Bowel Diseases. 2013.

[20] Perez-Brocal V *et al.* Clinical and Translational Gastroenterology. 2013.

[21] Barton ES *et al.* Nature. 2007.

[22] White DW *et al.* Blood. 2010.

[23] Yager EJ *et al.* Viral Immunology. 2009.

[24] Canny SP *et al.* Journal of Virology. 2014.

[25] Powell RD *et al.* The American Journal of Medicine. 1961.

[26] Roblin X *et al.* The American Journal of Gastroenterology. 2011.

It is also assumed that the difference in the immunological milieu between UC and CD is responsible for the lower rate of reactivation of CMV in CD compared to UC[27-29].

Another issue that remains to be clarified is the role of CMV in disease severity. Some reports and studies suggest that colonic superimposed CMV infection is associated with an increased rate of complications, namely toxic megacolon and need for surgery[30-32]. On the other hand, there are more recent papers suggesting that the viral load does not impact on clinical outcome[26, 33].

Although the appealing nature of this association, it has been difficult to prove if CMV is really a causative factor in the pathogenesis of severe colitis or whether it simply represents a surrogate marker of a severe or steroid-refractory disease.

At the present time there are several questions concerning CMV without a definitive answer: whether it is more prevalent in IBD population than in general population; if there is a significative difference in prevalence between CD and UC; whether viral load is higher in ulcerated compared to normal mucosa; if the presence of CMV DNA in the colonic mucosa determines the severity of the disease and has therapeutical implications.

Regarding EBV, the major concern is its association in immunocompromised patients with several malignancies namely Hodgkin´s disease, T/NK-cell lymphoma, Burkitt's lymphoma and gastric carcinoma. The implication of EBV on this malignancy risk translates from the transplant setting and its association with post-transplant lymphoproliferative disease (PTLD)[34]. In IBD, information regarding the role of EBV in disease course and prognosis is scant, due to the lack of a consensual and proved more specific method to define disease *vs* infection, and a greater focus on CMV infection. Increasing interest in EBV has derived from the published evidence of the increased risk of lymphoma in IBD, especially among young male patients under thiopurines. In immunosuppressed patients, this increased risk seems to be related to reactivation of a latent or a primary EBV infection[35]. There is the notion that EBV-positive cells can be found in the colonic mucosa of more than half of IBD patients, predominantly in the inflamed areas[36-38]. The reason for this high percentage is the increased number of infiltrating B-lymphocytes

[27] Nakase H *et al.* Digestive Diseases and Sciences. 2010.

[28] Knosel T *et al.* Pathology, Research and Practice. 2009.

[29] Takahashi Y *et al.* Diseases of the Colon and Rectum. 2004.

[30] Domenech E *et al.* Inflammatory Bowel Diseases. 2008.

[31] Kojima T *et al.* Scandinavian Journal of Gastroenterology. 2006.

[32] Kambham N *et al.* The American Journal of Surgical Pathology. 2004.

[33] Leveque N *et al.* Journal of Medical Virology. 2010.

[34] Green M *et al.* Am J Transplant. 2013.

[35] Beaugerie L *et al.* Dig Dis. 2009.

[36] Wakefield AJ *et al.* Journal of Medical Virology. 1992.

[37] Ryan JL *et al.* Digestive Diseases and Sciences. 2012.

[38] Spieker T *et al.* The American Journal of Pathology. 2000.

in the mucosa due to inflammation, and the increased EBV replication rate as a result of immunosuppression[39].

Several authors have studied the prevalence of EBV in IBD patients. In a work by Knosel T *et al*[28], EBV DNA detected by PCR was the second most prevalent agent in patients with CD, and not detected in the control group. This higher prevalence in CD patients compared to healthy individuals in a German population, was also reported by Spieker *et al*[38] and Ruther *et al*[40]. This evidence was not reproduced in a Belgian and French population[41] and the clinical relevance of EBV-positivity in colon cells remains unclear, either relatively to disease course and malignant complications.

A recent prospective study by Ciccocioppo *et al*[42] evaluated the prevalence of CMV and EBV in both blood and colonic mucosa, by quantitative PCR and immunohistochemistry. The authors concluded that quantitative real-time PCR was the best method to define disease. They found a higher prevalence of both CMV and EBV in refractory patients compared to non-refractory and the control group, a higher median mucosa viral load in refractory IBD, and a significantly higher viral DNA load in disease vs non-disease mucosa. Mucosal viral load positively correlated to the degree of endoscopic activity, both for EBV and CMV. No difference was found between median viral DNA levels of non-diseased mucosa and those of non-refractory IBD patients and controls.

A relevant finding was the determination of a cut-off value, above which viral related disease was considered, suggesting a closer follow-up and early recognition of patients at risk. Regarding the impact of therapy on viral prevalence, the authors found systemic steroid use a significant risk factor for EBV and CMV colitis, and no correlation with de use of immunosupressants. By contrast, our group found a higher prevalence of EBV in patients under infliximab, irrespective of associated use of other immunosupressors[43]. On the other hand we could not find any correlation between the number of copies in serum and therapeutic regimen or C-reactive protein (CRP) level. Despite this evidence, it remains to be established if and which IBD patients should be tested and followed in the long-term. In the post transplant context serial monitoring of EBV viral load is advocated, and preventive treatment of the infection can reduce the morbidity and mortality of

[39] Kumar S *et al*. The American Journal of Surgical Pathology. 2000.

[40] Ruther U *et al*. Hepato-Gastroenterology. 1998.

[41] Van Kruiningen HJ *et al*. APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica. 2007.

[42] Ciccocioppo R *et al*. World Journal of Gastroenterology. 2015.

[43] Magro F *et al*. Inflammatory Bowel Diseases. 2013.

EBV-related lymphoproliferative disorders in PTLD[44]. If this approach is to be implemented in IBD needs further evidence.

Another HHV related to gastrointestinal symptoms in immunocompromised patients is Human Herpes Virus-6. The seroprevalence in adulthood is estimated to exceed 95%. In IBD, data on HHV-6 is scarse. The importance of HHV-6 reactivation in immunocompromised patients is being recognized in the post-transplant population, being responsible for severe morbidity and sometimes graft lost. It has been identified as a possible trigger to other herpesvirus infections, especially CMV[45-48], being suggested that immunosuppressed patients harboring HHV-6 should be more closed monitored. As HHV-6 DNA has been identified in the mucosa of post-transplant patients with gastrointestinal symptoms, it was hypothesized that in IBD immunosuppressed patients it could be a trigger of relapse. Some studies have shown a high prevalence of HHV-6 DNA in the colon of both UC and CD patients[36, 49]. By contrast, archival tissue examination of CD patients using PCR technique found HHV-6 positivity in only 3.6% (2/56) of samples[28]. A prospective study by Sipponem T *et al*[50] evaluated the prevalence of CMV and HHV-6 antigens in the mucosa in endoscopically active and inactive CD and UC patients and in a non-IBD control population. CMV and HHV-6 antigenemia were also evaluated in IBD patients. In this study, the authors found a higher positivity for HHV-6 in both UC (45%) and CD (44%) patients. This number was comparable with that seen in solid organ transplant recipients[51]. Both virus were shown to be more prevalent in endoscopically active mucosa compared to endoscopically inactive segments, with a significant difference in HHV-6 antigen expression in more severe endoscopic disease (p=.042). One interesting finding was the simultaneous expression of both virus in patients under more than one immunosupressive medication and with endoscopically more severe disease (at least moderate disease). In IBD patients, clinical significance of this coexistence is unknown, but in transplant recipients the presence of HHV-6 in tissue has the potential to trigger other herpesvirus infections, especially CMV[45-48, 52]. Another feature that remains to be elucidated is the role of anti-TNF alpha agents in patients testing positive for these viruses, with some conflicting results. In this series, the CMV expression in the mucosa was more intense in patients under biologicals or cyclosporine, but the same was not proved for HHV-6.

[44] Comoli P *et al.* American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2007.

[45] Halme L *et al.* Clinical Infectious Diseases: an Official Publication of the Infectious Diseases Society of America. 2008.

[46] Halme L, *et al.* APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica. 2008.

[47] Mendez JC *et al.* The Journal of Infectious Diseases. 2001.

[48] DesJardin JA *et al.* The Journal of Infectious Diseases. 1998.

[49] Sura R *et al.* APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica. 2010.

[50] Sipponen *et al.* Scandinavian Journal of Gastroenterology. 2011.

[51] Razonable RR *et al.* American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2009.

[52] Lautenschlager I *et al.* Clinical Infectious Diseases: an Official Publication of the Infectious Diseases Society of America. 1998.

Until the present time, it remains to be proved with no margin of doubt if the changes observed in number and richness of virus in IBD is biologically and clinical relevant in terms of disease phenotype, behavior and response to therapy.

Imaging Modalities

Endoscopy

One of major difficulties in IBD is establishing the correct diagnosis. Being a chronic, lifelong condition, needing in the majority of patients of prolonged and intensive immunosuppression, an unequivocal diagnosis is mandatory. Despite typical, symptoms are not pathognomonic of either disease (CD or UC), and objective data are required to make the diagnosis. Ileocolonoscopy is the gold standard, allowing to rule out other conditions that may mimic IBD symptoms and to obtain tissue samples for histological assessment. It is accurate in differentiating CD from UC and has a role in evaluating disease extent and severity, serving as a prognostic tool.

Endoscopic scores developed to standardize reports of mucosal lesions in IBD, both in clinical trials and clinical practice. In CD, the first endoscopic score developed and validated was de Crohn´s disease endoscopic index of severity (CDEIS)[53]. This score proved to be complex and cumbersome, with limited application in clinical practice. In 2004 a simplified version of CDEIS, the simple endoscopic score for Crohn´s disease (SES-CD)[54] was proposed and validated. This score ranges from 0 to 56, and mucosal healing is defined as a SES-CD<3. In UC, the most commonly used endoscopic scores are the Mayo Endoscopic subscore[55] and the Ulcerative Colitis Endoscopic Index of Severity (UCEIS)[56], a more recent and validated score. Endoscopic remission is defined as a Mayo Endoscopic subscore of 0 or 1 but for UCEIS no cut-off has yet been defined. UCEIS has proved to be useful in predicting medium and long term outcomes in UC patients.

If initially mainly limited to establishing the diagnosis, nowadays the role of endoscopy has evolved tremendously, not only due to technical developments but also to a change in the treatment paradigm of IBD. The severity of endoscopic findings and the extent of the disease are known prognostic factors predicting response to therapy and need for

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[56] Travis SP *et al.* Gut. 2012.

surgery. During disease flares, endoscopic evaluation of the mucosa allows to exclude other causes of exacerbation, namely infections. If traditionally the clinical effectiveness of therapy was evaluated by clinical indices, nowadays it is known that these indices have clear limitations and do not correlate with mucosal findings or clinical outcomes in the long time. In recent years, with the advent of new molecules capable of inducing mucosal healing (MH) (assessed by endoscopy), this became the new treatment goal both for CD and UC. In several clinical trials, the achievement of mucosal healing has been associated with improved outcomes, such as lower rate of relapse, steroid free remission, fewer hospitalizations and surgeries. There is also some evidence in UC that healed mucosa may be associated with a decrease rate of dysplasia and colon adenocarcinoma. Endoscopy has become an important evaluation tool of response to therapy and before any treatment adjustment. The Selecting Therapeutics Targets in Inflammatory Bowel Disease (STRIDE) program[57] developed recommendations for potential treatment targets to be used in clinical practice. Both in CD and UC, endoscopic remission was agreed as a target, and therapy adjusted if this goal was not achieved in the reevaluation endoscopy.

The performance of endoscopy in IBD goes beyond being merely a diagnostic or prognostic tool because it allows therapeutic procedures. One major complication in CD is stricture formation, still the main reason for surgery (either strictureplasty or bowel resection). Small bowel strictures occur in approximately 25% of Crohn's disease patients, and colonic strictures in about 10%. Up to 50% of CD patients undergo surgical resection within the first 10 years of diagnosis[58]. After surgery, symptomatic recurrence is as high as 38% at one year, with need to repeat surgery that can lead to short bowel syndrome. Endoscopic balloon dilation (EBD) has become an accepted alternative to surgery, with overall favorable results in terms of safety, efficacy, and patient satisfaction. Being a bowel conserving procedure with high technical (73%-100%) and clinical success rate (64%-70%), and a low rate of adverse events (2%-6.4%), its major drawback is the high rate of recurrence. Re-dilation may be required in up to 20% and 50% by 1 and 5 years, respectively(59-61) with the same high success rate, supporting the evidence that repeated dilations do not reduce the procedural efficacy[62, 63].

[57] Peyrin-Biroulet L *et al.* The American Journal of Gastroenterology. 2015.

[58] Bernell O *et al.* Annals of Surgery. 2000.

[59] Morar PS *et al.* Alimentary Pharmacology & Therapeutics. 2015.

[60] Morini S *et al.* Digestive and Liver Disease: Official Journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2003.

[61] Thomas-Gibson S *et al.* European Journal of Gastroenterology & Hepatology. 2003.

[62] Chen M *et al.* Inflammatory Bowel Diseases. 2014.

[63] Atreja A *et al.* Journal of Crohn's & Colitis. 2014.

A recent pooled analysis reported a technical success, clinical success, long-term symptomatic and surgical recurrence rates of 89%, 81%, 48% and 29%, respectively[64]; these data are almost exclusively derived from retrospective cohort studies and may somewhat overestimate the actual benefit. In 2013, the European Crohn's and Colitis Organization stated that EBD was safe and effective and allowed surgery to be avoided in CD patients with anastomotic strictures[65]. The overall technical success rate in the meta-analysis performed by Hassan *et al* was 86% (71%-100%), while 41% of patients required repeated EBD allowing an overall long-term clinical efficacy (avoidance of surgery) rate of 58% during a median follow up of 33 months[66]. Navaneethan *et al.* reported in their systematic review that over a median follow-up period of 15-70 months, only 27% of patients required surgical interventions, and 44% of patients required only one dilation with long-term success[67]. Notably, median time duration until the requirement of surgery was observed to be 4-33 months[67] underlining the role of EBD as a bridge to surgery if needed, in a significant proportion of patients.

Risk factors associated with need for subsequent dilation or surgery have been inconsistent. Disease duration, smoking, presence of inflammation, disease activity and therapy at the time of dilation have been suggested as predictive factors of a successful procedure. In the paper by Bettenworth *et al*[64] only a stricture length ≤5cm was associated with a re-dilation and surgery free outcome. It has also been extensively demonstrated that EBD is equally effective and safe in dealing with de novo strictures compared to anastomotic strictures.

After resection, due to ongoing inflammatory activity, disease recurrence is common, at or above the anastomosis. Endoscopy plays a key role in diagnosing and grading postoperative recurrence of CD. Endoscopic recurrence frequently occurs before symptoms develop, with a recurrence rate at 1 year of 83-93%. The Rutgeerts score is a grading score of endoscopic severity of disease recurrence at the ileocolonic anastomosis and neoterminal ileum. The grade of endoscopic recurrence has been shown to predict clinical recurrence. In its original paper, Rutgeerts demonstrated that 80% of patients with an endoscopic score of i0 or i1 at 1 year follow-up, remained stable at 3 years, while 92% of patients with a Rutgeerts score of i3-i4, had a

[64] Bettenworth D. Inflammatory Bowel diseases. 2017.

[65] Annese V *et al.* Journal of Crohn's & Colitis. 2013.

[66] Hassan C *et al.* Alimentary Pharmacology & Therapeutics. 2007.

[67] Navaneethan U *et al.* Surgical Endoscopy. 2016.

progressive and severe evolution at 3 years[68]. For the first time, in an era with no effective treatment for CD, it was postulated that "*therapeutic efforts should be directed towards those patients with severe progressive lesions over a long segment of the neoterminal ileum at endoscopy within 1year after surgery*". It was also suggested that postoperative management could aim at preventing early recurrent lesions and preventing the evolution of severe early lesions to symptomatic disease and its complications.

Despite its great popularity and wide use, the Rutgeerts score has not yet been validated, lacks interobserver agreement and has been a source of debate concerning the i2 subscore, which groups lesions at the anastomosis and neoterminal ileum together. With this in mind, in 2014, Gecse KB *et al* published in the form of an abstract, a work designed to evaluate the intra and interobserver agreement using the Rutgeerts and the Modified Rutgeerts score[69]. This Modified score subdivides the subgroup i2 in: i2a- lesions confined to the anastomosis, including anastomotic strictures, and i2b- more than 5 aphthous ulcers or larger lesions, with normal mucosa in between, in the neoterminal ileum, with or without anastomotic lesions. Endoscopic recurrence is considered only for a Modified Rutgeerts score ≥ i2b.

More than 20 years have gone since Rutgeerts´ publication and our understanding of the natural history of post-operative CD has improved. At present, there is major scientific evidence that supports early endoscopic evaluation of the neoterminal ileum in order to detect recurrence and adjust therapy. The POCER trial[70] demonstrated the short-term benefit of postoperative endoscopic evaluation and step-up treatment in case of endoscopic recurrence. This group of patients had a better prognosis at 18 months, with lower endoscopic recurrence, when compared to patients who received standard of care. A recent published technical review stated that endoscopic evaluation is recommended at 6 to 12 months postoperatively to evaluate for endoscopic recurrence with adjustment of medical therapy accordingly.

Endoscopy of the Small Bowel

Being a pan-enteric disease, CD can affect any segment of the gastrointestinal tract. In almost 2/3 of patients the small bowel is involved and up to 30% of newly diagnosed CD patients have disease limited proximally to the reach of ileocolonoscopy[71]. It is known that proximal

[68] Rutgeerts P *et al.* Gastroenterology. 1990.

[69] Gecse K *et al.* Gastroenterology.

[70] De Cruz P *et al.* Lancet. 2015.

[71] Annunziata ML *et al.* Digestive Diseases and Sciences. 2012.

small bowel (jejunal) involvement carries a worse prognosis with more relapses, hospitalizations and surgery[72, 73], so correct diagnose of disease extension and phenotype is mandatory.

The endoscopic evaluation of the small bowel includes video capsule endoscopy (VCE) and device-assisted enteroscopy (DAE). VCE has been widely used since early 2000, is a non-invasive and well-tolerated method, with a high diagnostic yield. Although in most cases the initial diagnosis of CD can be established only by ileocolonoscopy, the thorough evaluation of the entire digestive tract has implications in terms of prognosis and therapeutic decisions.

According to ECCO (European Crohn´s and Colitis Organization) guidelines[74], VCE should be reserved for patients in whom the clinical suspicion for CD remains high despite negative evaluation by ileocolonoscopy and radiological examination. Despite this, VCE is often used as a first line diagnostic tool after ileocolonoscopy in the suspicion of CD, as it is more accurate than sectional modalities in detecting lesions of the upper small bowel[75-77]. Its major advantage is an elevated sensitivity to detect superficial mucosal lesions, especially in the jejunum where the larger mucosal surface, with redundant folds and lesser lumen distension, increases the rate of false positives and negatives with transversal imaging methods.

In a recent meta-analysis[75] the diagnostic yield of VCE proved to be superior to ileocolonoscopy, small bowel follow-through and Computed Tomography Enterography (CTE) (incremental yield ranged from 22% to 47%, respectively). Only the 10% incremental yield compared to Magnetic Resonance Enterography (MRE) did not reach statistical significance. A meta-analysis from Uri Kopylov *et al*[78], demonstrated a similar diagnostic yield for detection of SB inflammation by CE, MRE and small intestine contrast ultrasonography (SICUS), both in suspected and established CD. However, CE consistently shows a superior accuracy for detection of proximal small bowel (SB) disease. This is of particular interest in the pediatric population, where proximal SB involvement as implications in management planning, and has a prognostic value being associated with a higher risk of surgery. A recent prospective study performed in CD patients in clinical remission diagnosed previously unknown proximal involvement in 51% of patients[79].

[72] Park SK *et al.* Journal of Clinical Gastroenterology. 2013.

[73] Flamant M *et al.* Inflammatory Bowel Diseases. 2013.

[74] Gomollon F *et al.* Journal of Crohn's & cColitis. 2017.

[75] Dionisio PM *et al.* The American Journal of Gastroenterology. 2010.

[76] Jensen *et al.* Clinical Gastroenterology and Hepatology: the Official Clinical Practice Journal of the American Gastroenterological Association. 2011.

[77] Pica R *et al.* Journal of Crohn's and Colitis.

[78] Kopylov U *et al.* Digestive and Liver Disease: Official Journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2017.

[79] Greener T *et al.* Journal of Crohn's & colitis. 2016.

Due to the need to standardize reports, two scoring systems were developed for CE. Both were prospectively validated and are able to quantify the inflammatory activity of CD, enabling the objective assessment of disease severity. The Lewis score[80], the more widely used, divides the small bowel into 3 equal parts - the tertiles - and has well established cut-offs to define remission, mild inflammation and moderate-severe inflammation. The Capsule endoscopy Crohn's disease activity index (CECDAI), or Niv score[81], divides the small bowel in 2 segments, proximal and distal, and the segmental and total score is calculated based on a formula. There are no define cut-off values to grade disease activity other than a score less than 4, that defines endoscopic remission.

If its positioning in disease diagnosis is still a matter of debate, much less has been investigated concerning VCE findings in therapeutic guidance and therapeutic changes[82-84]. Some studies addressed this issue, but with some limitations. In recent years, there have been some evidence of treatment modifications in CD patients after VCE, both in adults and pediatric populations, with short and long-term disease. If mucosal healing is the goal of treatment and does not correlate with symptoms, objective evaluation is needed. There are a limited number of published papers evaluating the role of VCE in mucosal healing assessment. In a meta-analysis published in 2017[85], mucosal healing assessed by VCE was found to be associated with clinical remission after a follow-up of 12 weeks to 24 months. Despite these promising results, currently there is no consensus definition of mucosal healing defined by VCE, and quantification of inflammatory activity by means of the validated Lewis score and CECDAI index is recommended.

Other indication with therapeutic implications is the detection of acute lesions in patients in clinical remission. Our group published a paper addressing the role of VCE in the therapeutic management of CD. In patients in clinical and biochemical remission, 36% changed therapy after VCE[86]. Immunosuppression and biological therapy was started in 28% and 5% respectively. In a prospective study evaluating 56 CD patients in clinical remission, the presence of active inflammation detected by VCE was a predictor of clinical relapse.

[80] Rosa B *et al.* Journal of Crohn's & Colitis. 2012.

[81] Niv Y *et al.* Endoscopy. 2012.

[82] Long MD *et al.* Inflammatory Bowel Diseases. 2011.

[83] Lorenzo-Zuniga *et al.* Digestive Diseases and Sciences. 2010.

[84] Gralnek IM *et al.* Digestive Diseases and Sciences. 2012.

[85] Niv Y. European Journal of Gastroenterology & Hepatology. 2017.

[86] Santos-Antunes J *et al.* World Journal of Gastroenterology. 2015.

An emerging indication for VCE is in postoperative evaluation, although the number of studies and patients included is very small. The 2 major advantages of VCE comparatively to ileocolonoscopy are the detection of lesions outside the scope of colonoscopy and patient´s preference[87-89]. As VCE carries a theoretical increased risk of impaction in this setting, at the present time, its use should only be considered if colonoscopy is unsuccessful or contraindicated or to evaluate proximal extension of the disease. There is only one pilot study[90] evaluating the performance of VCE in the diagnosis of postoperative recurrence of small bowel lesions in CD. In this study recurrence was defined as an increase of 100 points or more in Lewis Score at 6 months, compared to the VCE performed one month after surgery. A pilot study[91] including 22 CD patients previously submitted to surgery, assessed the value of pan-intestinal capsule endoscopy (PICE) for the detection of recurrence. The authors concluded that PICE is feasible and provides significant findings in the postoperative surveillance of CD patients with impact on clinical management.

The role of capsule endoscopy is still evolving in the management of CD patients, with its applicability expanding beyond just an initial diagnostic method. As CD patients will require multiple imaging procedures during the course of their disease, ease of use and reduction of patient's discomfort should always be considered as important. Patient preference may have a major impact on adherence with any monitoring strategy, and should be considered whenever possible.

Device assisted-enteroscopy (DAE) is an endoscopic method of examination of the small bowel with assisted progression in the lumen by a balloon or overtube. In the context of suspected Crohn´s disease, the diagnostic yield ranges between 22 % and 70%[92-94]. Its major advantage is the possibility of histological assessment to confirm the diagnosis or to exclude other conditions which mimic the appearance of Crohn's disease [92, 93, 95-98]. In patients with a high clinical index of suspicion for active Crohn's disease, DAE is more accurate than small-bowel barium contrast studies[99] and MRE[100, 101]. It has the disadvantages of being an invasive and time-consuming procedure.

[87] Bourreille A *et al.* Gut. 2006.

[88] Pons Beltran V *et al.* Gastrointestinal Endoscopy. 2007.

[89] Kono T *et al.* World Journal of Gastrointestinal Endoscopy. 2014.

[90] Cesarini M *et al.* Inflammatory Bowel Diseases. 2008.

[91] Hausmann J *et al.* Scandinavian Journal of Gastroenterology. 2017.

[92] Gay G *et al.* Gastrointestinal Endoscopy. 2007.

[93] Manes G *et al.* Surgical Endoscopy. 2009.

[94] Heine GD *et al.* Endoscopy. 2006.

[95] Seiderer J *et al.* Scandinavian Journal of Gastroenterology. 2007.

[96] Sunada K *et al.* World Journal of Gastroenterology. 2005.

[97] May A *et al.* Gastrointestinal Endoscopy. 2005;62(1):62-70.

[98] Prachayakul V *et al.* BMC Gastroenterology. 2013;13:103.

[99] Oshitani N *et al.* The American Journal of Gastroenterology. 2006; 101(7):1484-9.

[100] de Ridder L *et al.* Gastrointestinal Endoscopy. 2012.

[101] Takenaka K *et al.* Gastroenterology.

At the present date, according to recommendations of the European Society of Gastrointestinal Endoscopy (ESGE), the indication for DAE in the diagnosis of CD is limited to patients with noncontributory ileo-colonoscopy and with suspicion of Crohn's disease on small-bowel cross-sectional imaging modalities or VCE. Its major indication is as a therapeutic modality in bleeding, strictures dilation or foreign bodies retrieval[93, 99, 102]. Technical success rate in dilating strictures is reported to be between 60% and 80% and repeat endoscopic balloon dilation may be undertaken[103-105], but long-term outcomes are less well known. Complication rate, namely perforation, may be as high as 9%[103, 106-109]. An applicability that is being explored is its use in the assessment of mucosa healing, allowing evaluation of the activity of small bowel lesions as well as colorectal lesions by a single endoscopic procedure. A scoring system was developed, the modified Simple Endoscopic Score for Crohn's disease (mSES-CD), which includes assessment of the endoscopic activity of small bowel lesions. This score evaluates 2 ileal segments, 80 cm proximal to the ileocecal valve, and the total score ranges from 0-67. This score showed predictive value in clinical outcome, with patients with higher scores having lower surgery-free survival[110].

Enterography

Imaging modalities are of foremost importance in the management of inflammatory bowel disease. Although endoscopy is still the reference method in diagnosing disease, disease recurrence in the post-operative setting, and mucosal healing, further investigation is recommended.

Cross sectional imaging modalities, mainly magnetic resonance enterography (MRE) and computed tomography enterography (CTE), are complementary to endoscopy as they allow detection of transmural inflammation, penetrating and extraintestinal complications of CD, and evaluation of segments out of reach by conventional endoscopy. Both techniques are the current standards for assessing the small bowel, in terms of disease location, extension and activity, offering the opportunity to classify and stage disease. Radiological techniques have the advantage of identifying isolated transmural CD, not detected by endoscopic modalities.

[102] Lee BI *et al.* Gastrointestinal Endoscopy. 2005.

[103] Despott EJ *et al.* Gastrointestinal Endoscopy. 2009.

[104] Swaminath A *et al.* Inflammatory Bowel Diseases. 2008.

[105] Di Nardo G *et al.* Gastrointestinal Endoscopy. 2010.

[106] Fukumoto A *et al.* Gastrointestinal Endoscopy. 2007.

[107] Pohl J *et al.* European Journal of Gastroenterology & Hepatology. 2007.

[108] Hirai F *et al.* Digestive Endoscopy: Official Journal of the Japan Gastroenterological Endoscopy Society. 2014.

[109] Gill RS *et al.* Therapeutic Advances in Gastroenterology. 2014.

[110] Morise K *et al.* World Journal of Gastroenterology. 2015.

CTE consists of a high spatial resolution imaging of the small bowel with a multi-detector CT, after the ingestion of a neutral enteric contrast agent that allows luminal distension. The use of an enteric agent combined with an intravenous contrast agent permits to distinguish the different layers of bowel wall and the perienteric fat and mesentery. Artifacts due to bowel and respiratory movements are effectively eliminated as data is acquired in a single breath-hold. Most of the studies conducted to evaluate the performance of CTE for active CD have revealed a sensitivity range from 80-90%[111-114]. Disease activity is evaluated considering wall thickness, wall enhancement, mesenteric fat proliferation and densification, the presence of comb sign and enlarged lymph nodes. Conflicting data exist concerning the relationship between radiological signs of inflammation and disease activity. Some studies report a significant correlation between mural findings and biomarkers of disease activity[115] while others were not able to confirm these results[114, 116, 117]. Few data exist about the relationship between signs of perienteric inflammation and clinical or biochemical indices of activity. Lee et al first described the association of prominent perienteric or pericolic vasculature and active CD[118]. Colombel *et al* found a significant relationship between radiological findings of perienteric inflammation (increased fat density) and CRP[119]. Minordi *et al* evaluated both mural signs (parietal thickness, target sign or alternating rings of low and high density in the bowel wall) and extraenteric inflammation[120]. There was a positive correlation between the target sign and fibro-fatty proliferation and the Crohn´s Disease Activity Index (CDAI) and between wall thickness, comb sign and perienteric stranding and CRP. These inhomogeneous findings have limited the development of a scoring system that objectively evaluates CTE activity, defines activity vs remission and compares its evolution over time.

In clinical practice, the benefit of small bowel examination using an enterography technique has been explored. In a cohort of 357 patients with CD who underwent CTE, penetrating disease was identified in 20% of patients[121], representing a new finding in almost 2/3 of the patients. CTE also detected extraintestinal IBD in 18.8% of patients, being a new finding in 67.2%. In a prospective study involving 273 patients with

[111] Bodily KD *et al.* Radiology. 2006.

[112] Booya F *et al.* Radiology. 2006.

[113] Hassan C *et al.* International Journal of Colorectal Disease. 2003.

[114] Solem CA *et al.* Gastrointestinal Endoscopy. 2008.

[115] Maccioni F *et al.* Abdominal Imaging. 2000.

[116] Neurath MF *et al.* The American Journal of Gastroenterology. 2002.

[117] Schunk K *et al.* Investigative Radiology. 2000.

[118] Lee SS *et al.* AJR American Journal of Roentgenology. 2002.

[119] Colombel JF *et al.* Gut. 2006.

[120] Minordi LM *et al.* La Radiologia Medica. 2015.

[121] Bruining DH *et al.* Inflammatory Bowel Diseases. 2008.

established or suspected CD, CTE findings altered management plans in 1 of every 2 cases independent of clinical, serologic, and histological findings[122].

Its applicability goes beyond a diagnostic tool in assessing disease activity, extension and complications in a single time point. CTE may be and has been used in disease monitoring. In a preliminary study, Hara *et al* have reported the potential of CTE for longitudinal disease monitoring, noting its reliability to predict disease progression or regression[123]. A retrospective study, published in 2011[124], showed that about 2/3 of patients treated with anti-TNF alpha had a significant radiological response as assessed by serial CTEs. Minordi *et al* also found a good correlation between clinical and radiological evaluation of 45 CD patients, before and after therapy[120]. More recently, Deepak *et al* showed that achievement of complete or partial radiologic response at the first follow-up CTE/MRE after treatment decreased the risk for steroid usage by over 50% (hazard ratios: 0.37 [95% CI, 0.21–0.64] and 0.45 [95% CI, 0.26–0.79], respectively[125]. Complete response decreased the risk of subsequent hospitalization and surgery by over two-thirds (HR: 0.28 [95% CI, 0.15–0.50] and 0.34 [95% CI, 0.18–0.63], respectively). In addition, disease activity evaluated by CTE has proved to correlate with endoscopic activity[111, 119, 126], with the advantage of being a noninvasive method, better tolerated by patients and giving information about all the extension of the disease.

All these encouraging findings about the accuracy of CTE to evaluate disease response to therapy and its prognostic significance, gave rise to the suggestion that radiological response may be a more appropriate therapeutic target. The persistence of transmural inflammation despite mucosal healing, not diagnosed by endoscopy, often leads to structural bowel damage (BD) and intestinal complications conducting to surgery and subsequent increased bowel damage. It is known that BD is associated with a worse prognosis with a higher risk of hospitalization and surgery during follow-up.

[122] Bruining DH *et al.* Inflammatory Bowel Diseases. 2012.

[123] Hara AK *et al.* AJR American Journal of Roentgenology. 2008.

[124] Bruining DH *et al.* Clinical Gastroenterology and Hepatology: the Official Clinical Practice Journal of the American Gastroenterological Association. 2011.

[125] Deepak P *et al.* The American Journal of Gastroenterology. 2016.

[126] Sakurai T *et al.* European Journal of Radiology. 2017.

Even though MRE is attempting to replace CTE, due to concerns regarding radiation exposure, CTE is cheaper, more readily accessible, faster, with higher spatial resolution and better tolerated by patients. CTE may even be superior to MRE in terms of image quality and interobserver agreement[127]. Nowadays there are several strategies available to reduce radiation dose exposure, with no compromise of diagnostic accuracy.

[127] Qiu Y *et al.* Alimentary Pharmacology & Therapeutics. 2014.

The Lémann Score
(The Crohn's Disease Digestive Damage Score)

One of the major limitations in changing the course of CD is the delay between onset of symptoms and diagnosis. This allows BD to progress and at the time of first disease assessment the magnitude of BD is consistent with several years of disease activity, being irreversible and conducting to intestinal resection.

The clinical and endoscopic scores available to estimate disease severity only assess inflammation at a specific time point and do not allow physicians to evaluate the cumulative structural bowel damage and thus do not capture the progressive, destructive course of the disease. There have also been identified several prognostic factors at diagnosis associated with a worse outcome[128] (steroid-dependency, perianal disease, extensive small bowel disease, rectal involvement, extra-intestinal manifestations, young age, ileal disease, penetrating behavior), increased risk of surgery and hospitalization. Data from rheumatology shows that the only effective way to slow disease progression, improve the long-term benefits of therapy and alter the natural history of disease is intervening before tissue damage occurs. In CD, even during periods of clinical and biochemical remission subclinical inflammation often persists and there is an evolution of disease phenotype to stricturing or penetrating disease[129, 130]. Surgical resection of bowel, many times the treatment of these complications, should be recognized as the ultimate manifestation of bowel damage[57]. Following surgery, this cycle often recurs, leading to progressive loss of intestinal function and disability.

Treatment with immunomodulators or anti-TNF agents in the first 2 years of disease diagnosis have proved to be associated with reduced risk of strictures, intestinal and perianal surgery and any complication, compared to its initiation after 2 years of diagnosis [131]. This increased amount of evidence has been changing the way we approach CD. Despite not yet translated in clinical guidelines, it is of good clinical

[128] Zallot C *et al.* Digestive Diseases. 2012.

[129] Louis E *et al.* Gut. 2001.

[130] Cosnes J *et al.* Inflammatory Bowel Diseases. 2002.

[131] Safroneeva E *et al.* Alimentary Pharmacology & Therapeutics. 2015.

practice to stratify patients according to their risk of disease progression. In those with a high risk, more "effective/aggressive" therapeutic strategies and strict follow-up monitoring should be provided in order to try to change disease course and prevent structural bowel damage.

The Lémann Index (LI), using a combination of endoscopic and imagiological assessment of disease activity, is the first quantitative tool that measures the cumulative bowel damage by using resections and the extent and severity of lesions in the digestive tract of CD patients[132]. The development of this score aimed at the possibility to measure cumulative bowel damage at a specific time point in patients´ evolution and its progression over time; identify patients with CD at high (or low) risk of rapid damage progression; and assess the impact of treatment strategies on the progression of CD. In the near future, the LI calculation may play an important role in CD management. A recent paper from G. Fiorino *et al* evaluated the prognostic value of the LI in early CD[133]. In this study, the presence of BD at diagnosis, assessed by the LI, was associated with a higher risk of hospitalization and surgery during follow-up (median 4.9 years). The authors also found that disease activity, evaluated by MaRIA index, had little or no role in predicting disease course. Taking all these evidences together, it is growing the assumption that maybe we should explore this new therapeutic endpoint: transmural healing (TH). At present there are few papers focusing on TH, with a limited number of patients[134-136]. TH seems to be related to MH and disease duration, with higher response rates to anti-TNF alpha agents in early stages of the disease.

On the basis of this findings, we can assume that an early effective intervention in the course of the disease, with disease modifying agents, translates into MH and TH. As both end points correlate with each other, cross-sectional modalities could be used to monitor disease, lowering the need for repeated colonoscopies.

[132] Pariente B *et al.* Gastroenterology. 2015.

[133] Fiorino G *et al.* Journal of Crohn's & Colitis. 2017.

[134] Ordas I *et al.* Gut. 2011.

[135] Rutgeerts P *et al.* Gastroenterology. 2012.

[136] Castiglione F *et al.* Digestive and Liver Disease: Official Journal of the Italian Society of Gastroenterology and the Italian for the Study of the Liver. 2017;49(5):484-9.

Fecal Biomarkers

Over the last 20 years, progress has been made in the identification, quantification, and validation of a range of fecal biomarkers of inflammation. Conceptually, fecal markers would be more sensitive and specific in IBD due to some of their particularities. As the fecal stream is in direct contact with the inflamed mucosa, they are specific for intestinal inflammation and their concentration is assumed to reflect the extent and severity of the inflammation present and to promptly respond according to a decrease or increase in the inflammatory burden. They have gained a major role in IBD management in the last years due to their noninvasive benefits which gives them the possibility to be repeated as needed. Their applicability includes the differential diagnosis between inflammatory and functional disease, selecting patients to further work-up; the prediction of relapse, aiding therapeutic decision-making, and informing patient prognosis.

Calprotectin (FC), a member of the S-100 family of proteins, is a 36-kDa calcium-binding and zinc-binding protein complex constituting up to 60% of neutrophil cytosol protein that is released upon neutrophil activation[137]. It has also been described as a damage-associated molecular pattern (DAMPs) molecule. DAMPs molecules may be released by necrotic cells or secreted by activated inflammatory cells and are considered to function as a communication mechanism between innate and adaptive immunity to regulate immune function[138]. FC excretion reflects increased neutrophil migration into the gut lumen through an inflamed mucosa, but despite being specific for gut inflammation, it is not disease specific. FC has proved to provide a clear distinction between inflammatory disease, healthy controls, and functional bowel disorders[139-142]. Apart IBD, it is increased in non-steroidal anti-inflammatory drug enteropathy, pancreatic insufficiency, alcoholic enteropathy, and colorectal cancer.

Lactoferrin (FL), a 76-kDa iron-binding protein, similarly to FC is neutrophil derived, being the main component of secondary granules that degranulate during the inflammatory process[143]. Several authors reported a high sensitivity and specificity of FL in differentiating IBD from IBS, with sensitivities ranging from 78% to 88%, and specificities

[137] Theede K *et al.* Inflammatory Bowel diseases. 2016.

[138] Garcia-Sanchez V *et al.* Journal of Crohn's & Colitis. 2010.

[139] van Rheenen PF *et al.* BMJ. 2010.

[140] Licata A *et al.* Journal of Clinical Gastroenterology. 2012.

[141] Roseth AG *et al.* Scandinavian Journal of Gastroenterology. 1992.

[142] Tibble J *et al.* Gut. 2000.

[143] Abraham BP *et al.* Gastroenterology Clinics of North America. 2012.

between 85% and 100%. There is evidence that FL can be used in pediatric population and that FL elevation occurs prior to clinical relapse[144, 145]. Lactoferrin proved to correlate with clinical activity index both in CD and UC and with histological inflammation[146]. Some authors found a significant correlation between FL and CDEIS, being able to predict mucosal healing and response to anti-TNF alfa therapy[147, 148]. Patients with increased levels of FL were also at higher risk of postoperative recurrence compared to those with normal levels[149-151]. Despite this encouraging results FL has not been yet validated as extensively as FC.

There is also a large amount of evidence on the ability of FC in predicting endoscopic disease activity in IBD, both in CD and UC. In CD, CDEIS correlated better with FC than with CDAI[152]. Different studies have found different cut-off values of FC predictive of endoscopic remission and grading disease severity[152-154], limiting the comparison between studies and the establishment of a definitive and universal cut-off value. There is also some evidence suggesting a better correlation between the FC levels and endoscopic activity when the disease has ileocolonic or colonic involvement than when it is only ileal[153]. Other studies have also found a good correlation between FC levels and the degree of disease activity detected by magnetic resonance enterography[155] and small bowel inflammation score in capsule endoscopy (Lewis score)[156].

In UC, endoscopic disease activity correlates better with FC than with blood biomarkers or the clinical activity index[157]. Similarly to CD, different cut-off values of FC were able to discriminate among different grades of endoscopic severity[157]. Notably, histologic features of inflammation can be identified by FC measurements, with a significantly higher average level in patients presenting active histologic inflammation[158]. The clinical and prognostic implication of this finding is still a matter of debate, although several papers have found an increased risk of relapse in patients presenting histological activity despite endoscopic remission.

In patients in clinical remission, serial monitoring of FC is able to detect subclinical mucosal inflammation and identify patients at risk of

[144] Walker TR *et al.* Journal of Pediatric gastroenterology and nutrition. 2007.

[145] Vrabie R *et al.* Gastroenterology & Hepatology. 2014.

[146] Vieira A *et al.* BMC Research Notes. 2009.

[147] Sipponen T *et al.* Inflammatory Bowel Diseases. 2008.

[148] Jones J *et al.* Clinical Gastroenterology and Hepatology: the Official Clinical Practice Journal of the American Gastroenterological Association. 2008.

[149] Scarpa M *et al.* Diseases of the Colon and Rectum. 2007.

[150] Lamb CA *et al.* The British Journal of Surgery. 2009.

[151] Ruffolo C *et al.* Journal of Gastrointestinal Surgery: Official Journal of the Society for Surgery of the Alimentary Tract. 2010.

[152] D'Haens G *et al.* Inflammatory Bowel Diseases. 2012.

[153] Lobaton T *et al.* Journal of Crohn's & Colitis. 2013.

[154] Schoepfer AM *et al.* The American Journal of Gastroenterology. 2010.

[155] Cerrillo E *et al.* Inflammatory Bowel Diseases. 2015.

[156] Koulaouzidis A *et al.* Digestive Diseases and Sciences. 2016.

[157] Schoepfer AM *et al.* Inflammatory Bowel Diseases. 2013.

[158] Guardiola J *et al.* Clinical Gastroenterology and Hepatology: the Official Clinical Practice Journal of the American Gastroenterological Association. 2014.

relapse[159, 160]. FC has also demonstrated to be a good tool in evaluating response to therapy with anti-TNF alpha[161, 162].

Patients with low FC levels were associated with good response to therapy and long-term remission while patients with higher FC levels relapse in the short-term. Once more, different cut-off values were found to associate with the probability of relapse and remission. The same way CRP was found to be predictive of response to treatment, FC changes after initiation of a new therapy in active disease correlated with treatment response[163]. A decrease on FC concentration at week 2 in UC patients undergoing infliximab induction therapy, correlates with endoscopic remission at week 10[164].

[159] Costa F *et al.* Gut. 2005.

[160] D'Inca R *et al.* The American Journal of Gastroenterology. 2008.

[161] Ferreiro-Iglesias R *et al.* Journal of Clinical Gastroenterology. 2016; 50(2):147-51.

[162] Ferreiro-Iglesias R *et al.* Scandinavian Journal of Gastroenterology. 2016.

[163] Sands BE. Gastroenterology. 2015.

[164] De Vos M *et al.* Journal of Crohn's & Colitis. 2012.

Fecal Biomarkers in the Postoperative Setting

Surgery, albeit not a curative option for CD, is frequently needed in its management. Postoperative recurrence is common, with endoscopic lesions preceding the development of clinical symptoms. It is known that patients with more severe endoscopic lesions after surgery, will have a greater probability of future clinical recurrence[68, 165]. This evidence has been the rational for an early detection of postoperative recurrence and subsequent intervention. The POCER trial has recently demonstrated that treatment should be personalized and based on the risk of recurrence[166].

Today, ileocolonoscopy is the gold standard to monitor recurrence but it has some inconveniences and it is questionable its timely performance in real-world clinical practice. It is desirable to develop a simpler, noninvasive, more acceptable and cheaper alternative to diagnose early postoperative recurrence. Based on the good diagnostic and monitoring performance of fecal markers in IBD, recent studies have focus on the utility of FC and FL in the postoperative setting[167-174].

In a recent meta-analysis published in 2015, including 10 studies and 613 postoperative CD patients, the pooled sensitivity and specificity for assessing endoscopic recurrence was 0.82 (95% CI, 0.73-0.89) and 0.61 (95% CI, 0.51-0.71), respectively[175]. The overall positive and negative likelihood ratios was 2.11 (95% CI, 1.68-2.66) and 0.29 (95% CI, 0.197-0.44), respectively. As endoscopic recurrence is used to predict clinical recurrence, and a significant correlation between fecal markers and endoscopic score has been shown, it is assumed that fecal markers can have a role in predicting clinical recurrence after surgery in CD. In a series of recent studies, FC and FL levels were significantly higher in patients with endoscopic recurrence than in those in remission, with a better correlation with endoscopic activity than serological biomarkers. A recent study by Yamamoto *et al* also found that consecutive monitoring of FC after the initial ileocolonoscopy in patients with no endoscopic recurrence is useful in their management[174]. An

[165] Yamamoto T *et al.* United European Gastroenterology Journal. 2013.

[166] De Cruz P *et al.* Alimentary Pharmacology & Therapeutics. 2015.

[167] Wright EK *et al.* Gastroenterology. 2015.

[168] Boschetti G *et al.* The American Journal of Gastroenterology. 2015.

[169] Hukkinen M *et al.* Journal of Pediatric Surgery. 2016.

[170] Lopes S *et al.* Inflammatory Bowel Diseases. 2016.

[171] Wright EK *et al.* Inflammatory Bowel Diseases. 2016.

[172] Herranz Bachiller MT *et al.* Scandinavian Journal of Gastroenterology. 2016.

[173] Garcia-Planella E *et al.* Inflammatory Bowel Diseases. 2016.

[174] Yamamoto T *et al.* Therapeutic Advances in Gastroenterology. 2016.

[175] Qiu Y *et al.* Inflammatory Bowel Diseases. 2015.

increase in FC levels indicates the need to repeat ileocolonoscopy, while sustained low FC levels predicts a low risk of endoscopic recurrence, avoiding an unnecessary invasive endoscopic examination.

The drawback that limits the generalized use of these markers in clinical practice and in disease monitoring, is the large quantitative difference between the different assays, limiting its use interchangeably and making impossible to compare studies and standardize cut-offs. In the POCER study, a FC level greater than 100 mg/g indicated endoscopic recurrence, while in others, a cutoff value of 200 mg/g was used as predictor of recurrence[153, 176].

Much is still unsolved regarding fecal markers. It is assumed that fecal biomarkers present a considerable day-to-day variability, are not specific for IBD and their levels vary with age, making difficult to establish a unique cut-off value. Their utility in proximal small bowel disease has yet to be fully elucidated. In contrast, there is an increasing amount of evidence suggesting that FC can be used as a marker of disease recurrence after surgery and correlates with histological activity. The benefit of combining different fecal markers has not yet been irrefutably proven, with some authors suggesting that a composite index may enhance test utility, while others have not shown additional benefit in this combination[177-179]. It is necessary to determine the appropriate time interval for monitoring disease activity in different clinical contexts, and to standardize the assays used in order to define the most suitable cutoff levels to define disease activity, response to therapy and mucosal healing.

In summary, fecal markers have emerged as surrogate markers of mucosal inflammation/healing, even though the predictive value of uniform thresholds at an individual level has not been clearly demonstrated. More important than an isolated measurement is to have serial determinations that allow physicians to evaluate response to therapy, anticipate flares and adjust interventions.

[176] Orlando A *et al.* European Review for Medical and Pharmacological Sciences. 2006.

[177] Schoepfer AM *et al.* Diseases of the Colon and Rectum. 2007.

[178] Schoepfer AM *et al.* Inflammatory Bowel Diseases. 2008.

[179] Judd TA *et al.* Journal of Gastroenterology and Hepatology. 2011.

Aims

The specific aims of this Thesis were:

1. To evaluate the prevalence and viral load of Epstein–Barr virus, Cytomegalovirus, and Human Herpes virus 6 in blood and mucosa of adult patients with endoscopic active IBD. We intended to determine if the prevalence of these ubiquitous virus was higher in IBD patients compared to healthy controls, and to assess if virus prevalence was influenced by different therapeutic regimens. Associated objectives were to find a possible correlation between mucosal disease severity and viral load.

2. To evaluate the accuracy and best cut-off value of FC and FL in diagnosing endoscopic recurrence in CD patients submitted to resection surgery, using the Modified Rutgeerts score compared to the Rutgeerts score. We also intended to compare fecal markers to a clinical disease activity index and serum biomarkers in predicting endoscopic recurrence.

3. To assess the accuracy of FC and FL in predicting disease recurrence in asymptomatic CD patients presenting with anastomotic strictures, and selecting patients to endoscopic balloon dilation.

4. To evaluate the incidence of anastomotic strictures after intestinal resection in Crohn's disease, to demonstrate long-term efficacy and safety of endoscopic balloon dilation in CD strictures and its impact on the diagnosis of subclinical postoperative endoscopic recurrence.

5. To explore the correlation between endoscopic disease activity, fecal markers levels and CTE findings of inflammatory activity at diagnosis and one year after immunosuppressive therapy. We also aimed to determine the best cut-off value of FC to predict endoscopic remission and which CTE findings should be looked for when trying to define disease remission.

6. To evaluate the impact of video capsule endoscopy findings in the management of asymptomatic CD patients.

Results - Publications

Looking into Enteric Virome in Patients with IBD: Defining Guilty or Innocence?

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Background: Although there is some evidence suggesting that certain viruses may be involved in the onset of inflammatory bowel disease (IBD), data regarding viral prevalence and viral load in blood and mucosa of patients with IBD are scarce. The main aim of this study is to evaluate the prevalence and viral load of common Epstein–Barr virus (EBV), cytomegalovirus (CMV), and human herpes virus 6 in blood and mucosa of adult patients with endoscopic active IBD.

Methods: From January to December 2014, ulcerative colitis and Crohn's disease patients with active endoscopic disease were consecutively enrolled. Subjects undergoing colonoscopy for colorectal cancer screening served as healthy controls (HCs). Paired blood and mucosal samples from each patient and HC were collected for EBV, CMV, and human herpes virus 6 quantitative real time polymerase chain reaction assessment of the viral load.

Results: One hundred forty-five subjects were included; 95 IBD patients with active endoscopic disease (43 ulcerative colitis and 52 Crohn's disease) and 50 healthy subjects. CMV and EBV DNA were detected more frequently in the mucosa of patients with IBD compared with HCs (CMV $P = 0.017$; EBV $P < 0.001$), irrespective of IBD type. The frequency of human herpes virus 6 DNA detection both in the blood and in the mucosa did not differ between patients with IBD and HCs. EBV median viral load was similar in the inflamed and noninflamed mucosa was not affected by the use of immunomodulators and/or anti–tumor necrosis factor alpha agents, and did not correlate with endoscopic disease activity.

Conclusions: EBV, and to a lesser extent CMV, were more prevalent in patients with IBD than in HCs. Mucosal viral load was not influenced by the therapeutic regimen, did not differ between inflamed and noninflamed mucosa, and did not seem to be influenced by the endoscopic activity of the disease, suggesting that EBV may be more involved in the onset of IBD than in its severity and clinical evolution.

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Key Words: inflammatory bowel disease, pathogenesis, human herpes virus 6, cytomegalovirus, Epstein Barr virus

The mechanisms underlying the pathogenesis of inflammatory bowel disease (IBD) are not fully understood.[1] Although the recent advent of genome-wide association studies have unambiguously implicated several genes/loci, these do not explain the risk in full.[2] Several environmental factors have also been linked to the pathogenesis of IBD. Akin to cigarette smoking, antibiotics use and dysbiosis of the bacterial microbiome, there is evidence pointing to the role of certain viruses in IBD onset.[1,3] Importantly, in animal models, certain eukaryotic viruses have been shown to interact with IBD risk genes.[4]

Very little data exist regarding viral prevalence and viral load among patients with IBD. The most investigated viral candidates have been the cytomegalovirus (CMV), the Epstein–Barr virus (EBV), and the measles virus.[5–7] Both CMV and EBV, members of the *Herpesviridae* family, are acquired early in life, usually asymptomatic, and remain latent lifelong in healthy people.[8] It is well known that under immunosuppression these viruses may give rise to symptomatic infections.[9] IBD has been associated with a higher prevalence of EBV and CMV infection, being disputed whether they are really involved in the pathogenesis of the disease, associated with disease flares, complications, and response to therapy or are innocent bystanders.[5,6] On the other hand, the increasing use of immunosuppressive therapies, associated with an increased risk of opportunistic infections[10] had led to a recrudescence of interest on their role on IBD. Despite all the evidence linking EBV and CMV to IBD, the definitive role of these viruses in IBD is still a topic of ongoing

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TABLE 1. Clinical and Demographic Characteristics

Controls	n = 50
Age, mean, yrs (SD)	49.8 ± 15.9
Man, n (%)	25 (50.0)
CD	**n = 52**
Age, mean, yrs (SD)	37.1 ± 14.4
Man, n (%)	30 (57.7)
Age at diagnosis, n (%)	
A1 (≤16 yrs)	7 (13.4)
A2 (17–4 yrs)	41 (78.9)
A3 (>40 yrs)	4 (7.7)
Disease location, n (%)	
L1 (ileal)	14 (26.9)
L2 (colonic)	14 (26.9)
L3 (ileocolonic)	19 (36.5)
L1 + L4 (ileal + upper gastrointestinal tract)	3 (5.8)
L3 + L4 (ileocolonic + upper gastrointestinal tract)	2 (3.9)
Disease behavior, n (%)	
B1 (nonstricturing, nonpenetrating)	24 (46.1)
B2 (stricturing)	13 (25.0)
B3 (penetrating)	15 (28.9)
Concomitant treatment, n (%)	
5 ASA	18 (34.6)
Steroids	9 (17.3)
Immunomodulators (azathioprine/methotrexate)	30 (57.7)/2 (3.8)
Anti–TNF-α (adalimumab/infliximab)	11 (21.1)/9 (17.3)
Time under immunomodulators, mean, mo (SD)	59 ± 54
Time under anti–TNF-α, mean, mo (SD)	25 ± 17
Combination therapy	10 (19.2)
Endoscopic activity	
SES-CD 3–9	38 (73.1)
SES-CD ≥9	14 (26.9)
UC	**n = 43**
Age, mean, yrs (SD)	41.5 ± 13.5
Man, n (%)	23 (53.5)
Location, n (%)	
Proctitis	3 (6.9)
Left-sided colitis	22 (51.2)
Pancolitis	18 (41.9)
Concomitant treatment, n (%)	
5 ASA	39 (90.7)
Steroids	2 (4.7)
Immunomodulators (azathioprine/methotrexate)	17 (39.5)
Anti–TNF-α (adalimumab/infliximab)	10 (23.2)
Time under immunomodulators, mean, mo (SD)	43 ± 36
Time under anti–TNF-α, mean, mo (SD)	36 ± 23
Combination therapy	8 (18.6)
UC	n = 43
Endoscopic activity, n (%)	
Mayo score 2	33 (76.7)
Mayo score 3	10 (23.3)

5ASA, 5 aminosalicylates; SES-CD, simplified endoscopic score for CD.

controversy. human herpes virus 6 (HHV6) has also been studied, with some studies demonstrating the presence of HHV6 DNA in the inflamed mucosa of Crohn's disease (CD) and ulcerative colitis (UC) patients,[11] although no definitive evidence had been established between these 2 entities.[12]

The detection of these agents in blood or mucosa of patients with IBD remains a clinical challenge, being hard to distinguish between a superimposed infection and a disease relapse.[8] Recent data had highlighted the need and interest of quantifying mucosal viral load by real time polymerase chain reaction to achieve the correct diagnosis and establish proper management.[13,14]

This study aims to evaluate the prevalence and viral load of EBV, CMV, and HHV6 in the blood and mucosa (both inflamed and noninflamed) of adult patients with active endoscopic IBD; and to assess the influence of different therapeutic regimens on viral prevalence. To our knowledge, this is the first study to address simultaneously the detection of EBV, CMV, and HHV6 in the peripheral blood and mucosa of patients with IBD.

METHODS

Subjects

Consecutive adult patients followed-up at Centro Hospitalar Sao Joao (Porto, Portugal) with endoscopically active IBD were prospectively enrolled between January and December 2014. Patients were included if the following criteria were met: (1) presence of a definitive diagnosis of UC or CD based on accepted clinical, radiological, endoscopic, and histological criteria[15,16]; (2) requiring therapy with 5-aminosalicilates (5-ASA), steroids, azathioprine (AZA), methotrexate (MTX), infliximab (IFX), adalimumab (ADA), or any combination of the above; and (3) presence of endoscopic activity, defined by a Simplified Endoscopic Activity Score for CD (SES-CD) ≥ 3 or Mayo Endoscopic Score for UC ≥ 2.[17,18] Patients younger than 18 years, who were pregnant, or did not have endoscopically active disease at the time of the study were excluded. Sex-matched subjects undergoing colonoscopy for colorectal cancer screening who were not taking any of the above drugs served as healthy controls (HCs).

Clinical and demographic data, including age, sex, IBD location and behavior, endoscopic activity, and therapeutic

regimen, were collected. Paired peripheral blood and mucosal samples from each patient and HC were obtained at the time of colonoscopy for detection and quantification of EBV, CMV, and HHV6. For patients, mucosal samples were collected from both inflamed (erosions or ulcers) and endoscopically normal (at least 20 cm from the former) areas.

DNA Extraction

EBV nucleic acid extraction used a DNA blood Kit (Qiagen, Inc., Valencia, CA) protocol with EZ1 BioRobot (Qiagen Inc.) from a 200 μL of whole blood sample. For CMV and HHV6, plasma was obtained and 400 μL was extracted by EZ1 virus mini Kit protocol (Qiagen Inc.) using the same robot.

For mucosal samples, biopsies were minced and digested with proteinase K (Qiagen Inc.) at 56°C until completely homogenized and DNA was extracted with an automatic processing (QIAcube; Qiagen Inc.) and a spin column protocol (QIAmp DNA mini kit, Qiagen Inc.).

DNA Amplification

CMV, EBV, and HHV6 were amplified using genesig specific kits (Primer Design, Southampton, United Kingdom) and LightCycler 480 II (Roche diagnostics, Indianapolis, IN). To verify the accuracy and sensitivity of virus detection protocols, these kits were at first tested with external quality control panels (Quality control for Molecular Diagnosis, 2013). A calibration curve was obtained for each virus, running, in duplicate, successively diluted positive control samples.

To normalize those several targets quantitative results, tissue samples were submitted to a quantitative parallel PCR reaction targeting a region of the human apoprotein B (ApoB).[19] The DNA obtained from a sample with concentrated and quantified blood leukocytes extraction was diluted, and tested, in duplicate, for ApoB. Because ApoB is encoded by a single gene, the human cells in each tissue sample could be calculated and the virus quantification expressed in copies/10^5 cells.

Statistical Analysis

Categorical variables are described as absolute frequencies (n) and relative frequencies (%). Median and percentiles are used for continuous variables. When testing a hypothesis about continuous variables, nonparametric tests (Mann–Whitney or Kruskal–Wallis) were used as appropriate, taking into account normality assumptions and the number of groups compared. When testing a hypothesis about categorical variables a chi-square test and Fisher's exact test were used, as appropriate. All the reported P values are 2-sided, and P values of <0.05 were considered statistically significant. Statistical analysis was performed using the software SPSS v.20.0 data (SPSS Inc., Chicago, IL).

Ethical Considerations

This study was conducted according to the Declaration of Helsinki. The study protocols were approved by the Ethics Committee of Centro Hospitalar Sao, Porto, Portugal. Informed

TABLE 2. Comparison of Viral Agents Prevalence in Mucosa of Patients with IBD and Controls

	IBD Versus Controls, n (%)	P	CD Versus Controls, n (%)	P	UC Versus Controls, n (%)	P	CD Versus UC, n (%)	P
CMV	11 (12.1) versus 0 (0.0)	0.017	5 (10.4) versus 0 (0.0)	0.025	6 (14.0) versus 0 (0.0)	0.024	5 (10.4) versus 6 (14.0)	>0.999
EBV	61 (67.0) versus 9 (18.0)	<0.001	28 (58.3) versus 9 (18.0)	<0.001	33 (76.7) versus 9 (18.0)	<0.001	28 (58.3) versus 33 (76.7)	0.228
HHV6	39 (42.9) versus 14 (28.0)	0.102	17 (35.4) versus 14 (28.0)	0.064	22 (51.2) versus 14 (28.0)	0.096	17 (35.4) versus 22 (51.2)	0.432

consent was obtained from each patient and HC in accordance with the local institutional board regulations.

RESULTS

Patients Characteristics

A total of 95 IBD patients with active endoscopic disease and 50 HCs were enrolled. Forty-three patients had UC (mean age 41.5 ± 13.5, 53.5% male), whereas 52 patients had CD (mean age 37.1 ± 14.4, 57.7% male). Mean age of HCs was 49.8 ± 15.9 years old and 50% were male. At the time of the study, a total of 49 (51.6%) patients were under immunomodulators and 30 (31.6%) under anti–tumor necrosis factor alpha (TNF-α) agents. Eleven patients (9 with CD and 2 with UC) were on steroids at the time of the study. Among the 43 patients with UC, 3 had proctitis, 22 had left-side colitis, and 18 had pancolitis. Of the 42 patients with CD, 14 had ileal, 14 had colonic, 19 had ileocolonic, and 5 had upper gastrointestinal tract involvement (combined with ileal involvement in 2 and ileocolonic in 3). Demographic and clinical data are summarized in Table 1.

EBV Is the Most Prevalent Viral Agent in Patients with IBD

CMV and EBV DNA were detected more frequently in the mucosa of patients with IBD (CMV DNA 12.1%; EBV DNA 67.0%) compared with HCs (CMV 0%, $P = 0.017$; EBV 18.0%, $P < 0.001$). The prevalence of CMV and EBV DNA was similar in patients with CD and UC, and in both groups higher than in HCs (Table 2). Although CMV DNA was detected in the blood of only 1 patients with IBD and in no HC (P = NS), EBV DNA was detected in the circulation of 20% of both patients and HCs.

Regarding HHV6 DNA, 43% of patients with IBD tested positive in the mucosa (35% CD and 51% UC) and 2% in blood, whereas in HCs, 2% had detectable HHV6 in blood and 28% in the mucosa (P = NS for all comparisons).

When considering the prevalence of the different viruses regarding the presence of ulceration/inflammation of the mucosa, EBV and CMV DNA were more prevalent in inflamed mucosa compared with noninflamed areas, although this difference was only statistically different for EBV both in CD ($P = 0.025$) and UC ($P = 0.019$) (Table 3 & see Table 1, Supplemental Digital Content 1, http://links.lww.com/IBD/B537).

Immunosuppression Does Not Impact on CMV or EBV DNA Prevalence

Because immunosuppression could impact viral prevalence, we analyzed viral prevalence according to current treatment regimens in patients with IBD. The prevalence of CMV, EBV, and HHV6 DNA in both the blood and in the mucosa was similar in patients under therapy with 5-ASA compounds only compared with patients under immunomodulators (either AZA or MTX) and/or anti–TNF-α agents (see Table 2, Supplemental Digital Content 2, http://links.lww.com/IBD/B538).

EBV median viral load is similar in the inflamed and noninflamed mucosa and does not correlate with endoscopic disease activity.

An analysis comparing viral loads was also performed. Viral load of EBV was higher in inflamed mucosa of patients with CD compared with inflamed mucosa of patients with UC ($P = 0.010$). There were no significant differences in viral load of CMV and HHV6 in inflamed mucosa of patients with CD compared with inflamed mucosa of patients with UC (P = NS for all comparisons). There were also no significant differences in viral load of EBV and HHV6 in normal mucosa of patients with CD compared with patients with UC (Table 4). No significant differences were found regarding viral load of CMV, EBV, and HHV6 in ulcerated versus nonulcerated mucosa of patients with CD and UC (Table 5).

TABLE 3. Comparison of Viral Agents Prevalence Between Inflamed and Noninflamed Mucosa in Patients with IBD and Controls

	CMV, n (%)	*P*	EBV, n (%)	*P*	HHV6, n (%)	*P*
IBD versus controls						
Noninflamed mucosa	2 (2.3) versus 0 (0.0)	0.283	38 (43.2) versus 9 (18.0)	**0.003**	30 (34.1) versus 14 (28.0)	0.461
Inflamed mucosa	—	—	—	—	—	—
CD versus controls						
Noninflamed mucosa	1 (2.2) versus 0 (0.0)	>0.999	15 (33.3) versus 9 (18.0)	0.306	13 (28.9) versus 14 (28.0)	>0.999
Inflamed mucosa	—	—	—	—	—	—
UC versus controls						
Noninflamed mucosa	1 (2.3) versus 0 (0.0)	>0.999	23 (53.5) versus 9 (18.0)	<**0.001**	17 (39.5) versus 14 (28.0)	0.825
Inflamed mucosa	—	—	—	—	—	—
CD versus UC						
Noninflamed mucosa	1 (2.2) versus 1 (2.3)	>0.999	15 (33.3) versus 23 (53.5)	0.255	13 (28.9) versus 17 (39.5)	>0.999
Inflamed mucosa	5 (10.6) versus 6 (14.0)	0.631	26 (55.3) versus 29 (67.4)	0.849	13 (28.9) versus 17 (39.5)	>0.999

TABLE 4. Comparison of Median Viral Load Between Inflamed and Noninflamed Mucosa in Patients with IBD

	CMV Median, Copies/10^5 Cells, (IQR)	*P*	EBV Median, Copies/10^5 Cells, (IQR)	*P*	HHV6 Median, Copies/10^5 Cells, (IQR)	*P*
CD versus UC						
Noninflamed mucosa	—	—	1.0 (1.0–37.0) versus 1.0 (1.0–1.0)	0.860	7.5 (1.0–32.6) versus 1.0 (1.0–1.0)	0.157
Inflamed mucosa	1.0 (0.8–8.1) versus 3.77 (1.0–75.5)	0.429	3.6 (1.0–240.2) versus 1.0 (1.0–1.0)	0.01	1.0 (1.0–1.0) versus 1.0 (1.0–1.0)	0.928

IQR, interquartile range.

When we compared the median values with ranges of DNA copies of all viruses, as obtained by pooling together the data in the IBD group, from both inflamed and noninflamed mucosa, we did not find significantly different values between CD and UC. With respect to EBV and HHV6, when comparing IBD as a group or each disease separately with the control group, no differences were found (Table 6).

Last, to assess whether viral infections were associated with endoscopic activity, median viral loads were compared according to the SES-CD or the Mayo Endoscopic Score for UC (see Table 3, Supplemental Digital Content 3, http://links.lww.com/IBD/B539). The median EBV and HHV6 load was similar in patients with CD with a SES-CD 3 to 9 and those with a score ≥9. For patients with UC, those with a Mayo score 2 had a EBV, CMV, and HHV6 load that did not differ significantly from patients with a Mayo score 3.

DISCUSSION

In this study, we show that EBV, and to a lesser extent CMV, are prevalent agents in the mucosa of patients with IBD. Their prevalence does not seem to be influenced by the different immunosuppressive regimens. In addition, mucosal viral load does not differ between inflamed and noninflamed mucosa and does not correlate with endoscopic severity of both CD or UC, suggesting that viral agents, more strongly EBV, may play a role in the onset rather than in the severity of IBD.

Despite growing interest in the role of bacterial microbiome dysbiosis in intestinal inflammation,[1] less attention has been drawn to the role of the enteric virome in the pathogenesis of IBD. A recent study showed an alteration in the enteric virome of both CD and patients with UC that seemed to be the contributory factor to intestinal inflammation and bacterial dysbiosis.[20] It is well known that *Herpesviridae* family is characterized by chronic infection or viral latency after a primary infection, after which the virus may be reactivated. Despite clinical disease being rare in the immunocompetent patient, there are some doubts about the pathogenic effect of these agents under immunosuppressed states: whether they are responsible for the disease and their complications or they are only simple bystanders remains to be defined.[5]

Previous studies have attempted to determine the prevalence of different viruses in patients with IBD. Most of them are, however, marred by their retrospective nature, by the inclusion of surgery specimens, and for assessing only one virus at each time.[6,21–24] In this study, the detection of EBV, CMV, and HHV6 in the peripheral blood and mucosa of patients with IBD was addressed simultaneously for the first time. Herein, we show that the prevalence of EBV and CMV DNA in peripheral blood does not differ between HCs and patients with IBD, but a higher prevalence of EBV, and to a lesser extent of CMV, was found in the mucosa of patients with IBD compared with HCs. In accordance with the results reported by others, we also found EBV as the most prevalent agent, with 2/3 of patients with IBD showing

TABLE 5. Comparison of Median Viral Loads in Inflamed Versus Noninflamed Mucosa in Patients with CD and UC

	CD			UC		
	Inflamed Mucosa	Noninflamed Mucosa	*P*	Inflamed Mucosa	Noninflamed Mucosa	*P*
EBV						
Median, copies/10^5 cells, (IQR)	3.60 (1.00–240.20)	1.00 (1.00–37.00)	0.333	1.00 (1.00–1.00)	1.00 (1.00–1.00)	0.612
HHV 6						
Median, copies/10^5 cells, (IQR)	1.00 (1.00–1.00)	7.50 (1.00–32.60)	0.173	1.00 (1.00–1.00)	1.00 (1.00–1.00)	>0.999
CMV						
Median, copies/10^5 cells, (IQR)	1.00 (0.8–8.10)—	—	—	3.77 (1.00–75.5)—	—	—

E.Coli, *Eschiria Coli*; IQR, interquartile range.

positivity for EBV.[14] Also, noteworthy is the finding of a higher prevalence of viral DNA in intestinal mucosa compared with the peripheral blood. This may suggest a potential role for EBV and CMV in the immune disturbance present on intestinal mucosa; alternatively, in case of CMV, it may only reflect its known tropism for inflammatory cells.[25]

Despite previous studies focused on the role of CMV in UC,[21,23] in this study, we only found a higher prevalence in diseased mucosa for EBV, both in CD and UC. However, when analyzing the EBV viral load within the IBD group of patients, the median values were not found to be significantly higher in diseased versus nondiseased mucosa. The observation that EBV is equally found in inflamed and noninflamed mucosa, suggests its role as a trigger of IBD rather than causing a superimposed infection. Because of the inexistence of CMV DNA in normal mucosa, and the very low viral load in inflamed mucosa, no conclusion could be drawn regarding CMV infection.

The possible role of viruses as trigger(s) of IBD is further supported by the absence of correlation between the mucosal viral load and the degree of endoscopic activity. Even in normal mucosa, there were no significant differences in the median viral DNA levels in patients with IBD compared with the control group.

On the other hand, HHV6 seemed to have no role in the pathogenesis of IBD, as prevalence and viral load was similar between the IBD population and the control group, suggesting that not all viruses are associated with IBD pathogenesis.

In contrast to published evidence of a significantly increased risk of EBV and CMV reactivation under immunomodulator therapy,[6,26] we did not find any positive relation between both anti–TNF α and/or AZA/MTX use and viral prevalence. In a previous study by our group,[6] where we aimed to evaluate EBV prevalence in the blood of patients with IBD at remission and compare that with the general population, we found that reactivation of EBV is more frequent among patients with IBD and that therapeutic regimen did influence the prevalence of EBV. Although the methodology used to detect EBV DNA in both studies were the same, in the current study, we have included patients with endoscopically active disease only, and therefore, we were not able to reproduce such finding. In addition, although in the 2013 study the mean age of the control group was 35 years, in the current it was 49.8 years[6]; the age difference between the 2 studies was because of ethical constraints in obtaining intestinal mucosa from healthy subjects younger than 50 years. In line with the current study, there are studies suggesting that only steroids and anti–TNF-α agents are associated with EBV colitis, but not the use of immunosuppressants or the duration of therapy.[14]

In summary, we provide evidence that EBV, and to a lesser extent CMV, are prevalent in patients with IBD, and that their prevalence is not affected by different therapeutic regimens. In addition, mucosal viral load does not differ between inflamed and noninflamed mucosa, and does not seem to be influenced by the endoscopic activity of the disease. Taken together, these data

TABLE 6. Comparison of Viruses Median Viral Load in Mucosa of Patients with IBD (Inflamed + Noninflamed Mucosa) and Controls

	IBD Versus Controls, n (%)	*P*	CD Versus Controls, n (%)	*P*	UC Versus Controls, n (%)	*P*	CD Versus UC, n (%)	*P*
CMV	—	—	—	—	—	—	1.0 (0.8–8.1) versus 3.77 (1.0–1229.9)	0.304
EBV	1.0 (1.0–62.3) versus 1.0 (1.0–3.8)	0.641	3.6 (1.0–153.5) versus 1.0 (1.0–3.8)	0.251	1.00 (1.0–36.4) versus 1.0 (1.0–3.8)	0.816	3.6 (1.0–153.5) versus 1.00 (1.0–36.4)	0.067
HHV6	1.0 (1.0–21.9) versus 1.0 (1.0–5.2)	0.488	3.8 (1.0–36.8) versus 1.0 (1.0–5.2)	0.132	1.0 (1.0–1.0) versus 1.0 (1.0–5.2)	0.831	3.80 (1.0–36.8) versus 1.0 (1.0–1.0)	0.099

support a putative role of certain viral agents, most notably EBV, in IBD pathogenesis and suggest that their frequent detection is not associated with either disease severity or superimposed infection. A note of caution, however, should be made when trying to extrapolate the findings obtained from our patients' cohort that included patients with endoscopically active disease, a relatively high proportion of subjects under immunomodulators and/or anti–TNF-α agents, to IBD population in general. Larger studies are needed to address other potential caveats (i.e., age at disease onset) and make these investigations more readily applicable.

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ORIGINAL ARTICLE

Correlation Between Calprotectin and Modified Rutgeerts Score

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Background: Endoscopic recurrence after surgery for Crohn's disease (CD) is high, and it has important prognostic value. Crohn's disease will recur in the majority of patients after surgery. Fecal calprotectin (FC) and lactoferrin (FL) have attracted interest in the postoperative setting for predicting relapse. We have evaluated the accuracy of FC and FL in diagnosing endoscopic recurrence (ER) using the modified Rutgeerts score (MRS) compared with the Rutgeerts score (RS).

Methods: A series of consecutive patients who underwent ileocolonic resection for Crohn's disease were evaluated. Biomarkers, clinical indexes, and fecal markers were recorded on the day of ileocolonoscopy. ER was defined as a MRS $\geq$ i2b or a RS $\geq$ i2.

Results: Ninety-nine patients were included in this prospective cohort. The median time between surgery and colonoscopy was 87.5 months (IQR, 31–137). FC and FL levels were higher in patients with ER than in those in remission (Median FC, 196.5 μg/g [IQR, 96–634 μg/g] versus 42.1 μg/g [IQR 19–91.60 μg/g; $P < 0.001$]; Median FL, 23.27 μg/g [IQR 8.9–47.8 μg/g] versus 2 μg/g [IQR 0.9–7.26 μg/g; $P < 0.001$]). Using the MRS, 34% of patients presented with ER compared with 76% if the RS was used. The RS performed worse than the MRS with a decrease in sensitivity (74% versus 48% for FC and 85% versus 55% for FL) and in NPV (91% versus 33% for FC, and 90% versus 37% for FL). Furthermore, the accuracy of the MRS was higher than that of the RS (75% versus 55%).

Conclusions: Both FC and FL proved to correlate well with endoscopic findings in the evaluation of Crohn's disease after surgery. Both markers predicted recurrence with greater accuracy when the MRS was used. Fecal markers can be used to monitor disease recurrence after intestinal resection, with patients being selected to undergo further endoscopic evaluation.

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Key Words: Crohn's disease, endoscopy, biomarkers

One of the major drawbacks of surgical treatment of Crohn's disease (CD) is the high recurrence rate after bowel resection. It is assumed that almost 60% of patients will have endoscopic recurrence within 5 years of surgery, and of those, a third will have clinical recurrence.[1] Disease recurrence may be misdiagnosed if based solely on symptoms and serum markers (C-reactive protein [CRP]) of inflammation. Despite being sensitive in CD, they are rather nonspecific and frequently fail to detect endoscopic recurrence.[2–4] Furthermore, 20% to 25% of CD patients experiencing flares do not exhibit increased CRP due to genetic single-nucleotide polymorphisms in the CRP gene, which affect CRP production.[5,6] The clinical indexes used in CD, namely the Crohn's Disease Activity Index (CDAI) and the Harvey Bradshaw Index (HBI), have poor correlation with endoscopic findings in this setting.[7] Ileocolonoscopy has been considered the gold standard in diagnosing recurrence, which is defined as de novo appearance of mucosal lesions in the neoterminal ileum, proximal to ileocolonic anastomosis.[8] The Rutgeerts score[8] has been used to classify the endoscopic findings after surgery. In 2014, Gecse KB et al[9] published, in the form of an abstract, a work designed to evaluate the intrarater and interrater agreement using the Rutgeerts score and the modified Rutgeerts score. This modified score performed well with high interclass correlation coefficients, and the authors proposed its validation for clinical use. Nevertheless, due to its invasiveness and the need for bowel preparation, colonoscopy is not very well accepted by patients and not easily repeated. The need for an accurate noninvasive marker that could be sequentially carried out and correlated with endoscopic recurrence and its severity has led to a growing interest in fecal biomarkers.[10–13]

Calprotectin (FC) is a 36-kDa calcium-binding and zinc-binding protein complex constituting up to 60% of neutrophil cytosol protein that is released upon neutrophil activation.[14] FC

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excretion reflects increased neutrophil migration into the gut lumen through an inflamed mucosa, but despite being specific for gut inflammation, it is not disease specific. Lactoferrin (FL), a 76-kDa iron-binding protein, similarly to FC is neutrophil derived, being the main component of secondary granules that degranulate during the inflammatory process.[14] Both these proteins are remarkably stable and resistant to degradation, easily detected, and measured in feces by commercially available ELISA kits. Both markers have proved to reflect endoscopic disease activity in CD, predicting endoscopic inflammation and being a surrogate marker of mucosal healing.[10,11] Until recently, the studies addressing the role of fecal markers in the CD postoperative setting have been limited, with a small number of patients, with no correlation with endoscopic findings and with some conflicting results.[1,15–20] In 2015, Wright EK et al[21] published the first prospective, multicentric, randomized, controlled trial in the postoperative setting of CD, evaluating the accuracy of FC in reflecting the presence and severity of disease recurrence. In this work, FC measurement was sensitive enough to monitor for CD recurrence after resection, and it also had a high negative predictive value, that is, patients with normal FC did not have endoscopic evidence of recurrence.

In our study, we evaluated the accuracy of FC and FL in diagnosing endoscopic recurrence according to the modified Rutgeerts score and compared its performance with the Rutgeerts score and serum biomarkers of inflammation.

MATERIAL AND METHODS

Objectives

The primary objective of this work was to compare the accuracy of FC and FL in diagnosing endoscopic recurrence according to the modified Rutgeerts and Rutgeerts score and to define which endoscopic score is more accurate in predicting recurrence. Secondary end points were: to correlate the clinical disease activity index (HBI) with the endoscopic index (modified Rutgeerts score) and FC and FL levels; to compare fecal markers with markers of inflammatory activity (CRP) in predicting postoperative recurrence; to investigate how FC and FL correlate with each other in the postoperative setting; to assess which fecal marker is the best predictor of endoscopic recurrence; and to determine the best cutoff value for FC and FL in the prediction of relapse, in the postoperative setting.

Population

Ninety-nine adult (>18 yr) consecutive patients followed at our inflammatory bowel disease (IBD) outpatient clinic with a diagnosis of CD, who had undergone curative ileocolonic resection for disease complication from 1994 to 2013, were prospectively enrolled in this study. Patients were excluded if the neoterminal ileum had not been endoscopically evaluated if they had colonic active disease or if they had taken nonsteroidal antiinflammatory drugs in the month before the colonoscopy.

All patients gave informed written consent to participate in the study, which was approved by the ethics committee of our institution. Demographic data were collected prospectively from a database (http://www.gediibasedados.med.up.pt) created for patients with IBD. The analyzed information was: sex, date of birth, age at symptom or disease onset, disease location and behavior, smoking status, date of abdominal surgery, follow-up after ileocolonic resection, time since surgery and colonoscopy, and current and previous pharmacological therapy.

Methods

Clinical disease activity was assessed on the day of endoscopic examination according to the clinical criteria of the HBI. This index was chosen because it has proved to have a good correlation with the CDAI and it is a simplified, less cumbersome alternative to the CDAI, as it does not require a prospective 7-day data collection, and is more suitable for use in clinical practice.[7] The variables inquired were referred to the day before bowel preparation. Clinically inactive disease was defined as an HBI of less than 5. Blood tests included hemoglobin (Reference range [RF]: 12.0 to 16.0 g/dL), leukocytes (RF: 4–11 $\times$ 10^9/L), platelets (RF: 150–400 $\times$ 10^9/L), albumin (RF: 38–51 g/L), and CRP (upper limit of normal <3 mg/L). Blood samples were obtained on the day of endoscopic examination if no previous results were available from the last month. Blood results with ≤4 weeks were accepted if there had been no changes in symptoms, and if no changes in therapy had occurred during that time period. All patients were referred for colonoscopic evaluation under propofol sedation at the Gastroenterology Department of the Centro Hospitalar São João, Porto, between November 2012 and October 2014. For bowel cleansing, we used a polyethylene glycol bowel preparation solution (Klean Prep; Helsinn Birex Pharmaceuticals, Dublin, Ireland). All the procedures were performed by a single senior endoscopist (SL) with systematic intubation of the neoterminal ileum (if an anastomotic stenosis was present it was immediately dilated in order to gain access to the ileum). Postoperative disease activity of the neoterminal ileum was evaluated according to the Rutgeerts[8] and modified Rutgeerts score.[9] Endoscopic remission was defined as a modified Rutgeerts score of i0, i1, or i2a (i0—no lesions in the neoterminal ileum; i1—fewer than 5 aphthous lesions in the neoterminal ileum; i2a—lesions confined to the ileocolonic anastomosis, including anastomotic stenosis), and postoperative recurrence was defined as a modified Rutgeerts score ≥i2b (i2b—more than 5 aphthous ulcers or larger lesions, with normal mucosa in between, in the neoterminal ileum, with or without anastomotic lesions; i3—diffuse aphthous ileitis with diffusely inflamed mucosa; i4—large ulcers with diffuse mucosal inflammation or nodules or stenosis in the neoterminal ileum).

Stool samples were collected the day before beginning bowel preparation (preferably from the first stool in the morning) and then kept in the fridge until being brought to the hospital. For lactoferrin evaluation, stools were stored at −80°C upon arrival at the laboratory. For calprotectin, within a maximum of 7 days

after collection, stools were extracted in accordance with the manufacturer's instructions, using a "Faecal sample preparation kit" (Roche Diagnostics, Mannheim, Germany). Sample extracts were stored at −80°C until the assays were performed at the Department of Pharmacology and Therapeutics, Faculty of Medicine of the University of Porto, by a single operator (JA). Samples were thawed and analyzed using a commercially available quantitative enzyme-linked immunoassay test (IBD-Scan; Tech-Lab, Blacksburg, VA) for lactoferrin and fluoroenzyme immunoassay (EliA Calprotectin; Thermo Fisher Scientific, Freiburg, Germany) for calprotectin. The techniques for measurements of both fecal markers and the cutoff values followed the manufacturer's guidelines (7.25 μg/g for lactoferrin and 50 μg/g for calprotectin).

Statistical Analysis

Categorical variables were described as absolute frequencies (n) and relative frequencies (%). Median and percentiles were used for continuous variables.

When testing a hypothesis about continuous variables, nonparametric tests (Mann–Whitney or Kruskal–Wallis) were used as appropriate, taking into account normality assumptions and the number of groups compared. When testing a hypothesis about categorical variables, a chi-square test and Fisher's exact test were used, as appropriate. Spearman's rank-order correlation test (r_s) was used to assess any correlation between calprotectin and lactoferrin, and between both variables and continuous parameters. Receiver operator characteristics (ROC) for lactoferrin and calprotectin (sensitivity and specificity) were assessed by curve analysis as described. All the reported *P*-values were 2-sided, and *P*-values of <0.05 were considered statistically significant.

For estimating a proportion of 0.7 with an effect size of 0.20, the sample power is higher than 85%, for a confidence level of 95%. All data were arranged, processed, and analyzed with SPSS v.20.0 data (Statistical Package for Social Sciences).

RESULTS

Endoscopic Recurrence

From November 2012 to October 2014, a total of 99 consecutive patients were included in this study. The mean age at the time of colonoscopy was 45 ± 14 years, the median disease duration between diagnosis and resection was 38 months (IQR, 6.5–119), and the median time between surgery and colonoscopy was 87.5 months (IQR, 31–137). Demographic and clinical characteristics of the study population are shown in Table 1. Of the 99 patients included, only 9% had clinically active disease defined as an HBI ≥ 5.

Endoscopic scores were i0 + i1 in 24 patients, i2 in 51 patients (i2a in 41 and i2b in 10 patients), and 24 patients presented endoscopic scores of i3 and i4. Considering recurrent endoscopic disease to be evaluated as a modified Rutgeerts score ≥ i2b, 34% of patients presented with recurrent disease (Table 2).

TABLE 1. Demographic and Clinical Characteristics of the CD Patients at the Time of Colonoscopy (n = 99)

	n
Sex, n (%)	
Male	47 (47)
Female	52 (53)
Age at endoscopic evaluation (mean [SD], yr)	45 ± 14
Time between surgery-endoscopic evaluation (median [IQR], mo)	87.5 (31–137)
Montreal classification n (%)	
Age at diagnosis	
A1	10 (10)
A2	75 (76)
A3	14 (14)
Disease location	
L1	57 (58)
L2	6 (6)
L3	32 (32)
L1 + L4	3 (3)
L3 + L4	1 (1)
Disease behavior	
B1	5 (5)
B2	50 (51)
B3	44 (44)
Perianal disease	
Yes	22 (22)
Smoking	
Yes	22 (22)
Obstructive symptoms at colonoscopy	
Yes	14 (14)
Therapy at time of colonoscopy	
Mesalamine	35 (35)
Immunomodulators	63 (64)
Corticosteroids	7 (7)
Anti-TNFα	30 (30)
Harvey Bradshaw Index	
Remission (HBI < 5)	90 (91)
Mild disease (HBI 5–7)	8 (8)
Severe disease (HBI > 16)	1 (1)

HBI, Harvey-Bradshaw Index; IQR, interquartile range; SD, standard deviation; TNF, tumor necrosis factor.

If we had used the Rutgeerts score, 76% of patients would have presented with recurrent disease (Rutgeerts score ≥ i2). No patient presented with colonic active disease.

There was no significant association between the HBI and either of the endoscopic scores ($P = 0.575$ for MRS and $P = 0.417$ for RS). There was also no association between endoscopic recurrence (assessed by both scores) and elapsed time from surgery to examination ($P = 0.442$ for MRS and $P = 0.622$ for RS); age at

TABLE 2. Rutgeerts Score and Modified Rutgeerts Score for Endoscopic Recurrence of CD After Ileocecal Resection

Rutgeerts Score		Modified Rutgeerts Score	
	n (%)		n (%)
i0	19 (19)	i0	19 (19)
i1	5 (5)	i1	5 (5)
i2	51 (52)	i2a	41 (42)
		i2b	10 (10)
i3	11 (11)	i3	11 (11)
i4	13 (13)	i4	13 (13)
Endoscopic recurrence			
No—i0, i1	24 (24)	No—i0, i1, i2a	65 (66)
Yes—i2, i3, i4	75 (76)	Yes—i2b, i3, i4	34 (34)

presentation ($P = 0.152$ for MRS and $P = 0.316$ for RS); and disease behavior ($P = 0.779$ for MRS and $P = 0.634$ for RS). With regard to smoking habits, we found a significant association between active smoking and recurrent disease only when we used the RS for classifying recurrent disease ($P = 0.012$ for RS and $P = 0.274$ for MRS). Considering therapy at the time of colonoscopy, being on 5-ASA, on corticosteroids or on anti-TNFα agents was not significantly associated with endoscopic recurrence, assessed by both scores. The use of immunomodulators was associated with nonrecurrent disease only if we used the RS ($P = 0.028$ for RS and $P = 0.276$ for MRS) (Table 3).

There was no statistical significant difference between biomarkers (hemoglobin, CRP) and the HBI in patients with endoscopic remission or in patients with endoscopic recurrence assessed by both scores.

Modified Rutgeerts Classification and Fecal Biomarkers

With regard to fecal markers, we found a positive correlation between FC and FL ($r_s = 0.558$, $P < 0.001$), irrespective of endoscopic recurrence, using the MRS. If we consider the recurrent disease group, this correlation was found to be strong ($r_s = 0.729$, $P < 0.001$). Fecal concentration of calprotectin and lactoferrin was significantly higher in patients with endoscopic recurrence (≥i2b) in the neoterminal ileum than in those without endoscopic recurrence (≤i2a) (Median FC, 196.5 μg/g [IQR, 96–634 μg/g] versus 42.1 μg/g [IQR, 19–91.60 μg/g; $P < 0.001$]; Median FL, 23.27 μg/g [IQR, 8.9–47.8 μg/g] versus 2 μg/g [IQR, 0.9–7.26 μg/g]; $P < 0.001$). If we define endoscopic recurrence as a Rutgeerts score ≥ i2, the median and interquartile range concentrations of calprotectin and lactoferrin were also higher in patients with endoscopic recurrence (FC, 95.1 μg/g [IQR, 43.4–321 μg/g] versus 23.3 μg/g [IQR 9.85–66.7 μg/g; $P < 0.001$]; FL, 8.6 μg/g (IQR 3.02–38.66 μg/g) versus 1.28 μg/

TABLE 3. Baseline Patients Characteristics According to Endoscopic Recurrence (Modified Rutgeerts Score and Rutgeerts Score)

	Endoscopic Recurrence					
	Rutgeerts			Modified Rutgeerts		
	i0, i1, n (%)	i2, i3, i4, n (%)	*P*	i0, i1, i2a, n (%)	i2b, i3, i4, n (%)	*P*
Age at diagnosis			0.316			0.152
A1	1 (4)	9 (12)		7 (11)	3 (9)	
A2	21 (88)	54 (72)		52 (80)	23 (68)	
A3	2 (8)	12 (16)		6 (9)	8 (23)	
Disease behavior			0.634			0.779
B1	2 (8)	3 (4)		4 (6)	1 (3)	
B2	13 (54)	37 (49)		33 (51)	17 (50)	
B3	9 (38)	35 (47)		28 (43)	16 (47)	
Smoking			0.012			0.274
Yes	1 (4)	21 (28)		13 (20)	9 (26)	
Former	9 (38)	12 (16)		17 (27)	4 (12)	
Therapy at time of colonoscopy						
Mesalamine	6 (25)	29 (39)	0.327	21 (32)	14 (41)	0.507
Immunomodulators	20 (83)	43 (57)	0.028	44 (68)	19 (56)	0.276
Anti-TNF	5 (21)	25 (33)	0.313	16 (25)	14 (41)	0.109
Corticosteroids	2 (8)	5 (7)	>0.999	4 (6)	3 (9)	0.689

TNF, tumor necrosis factor.

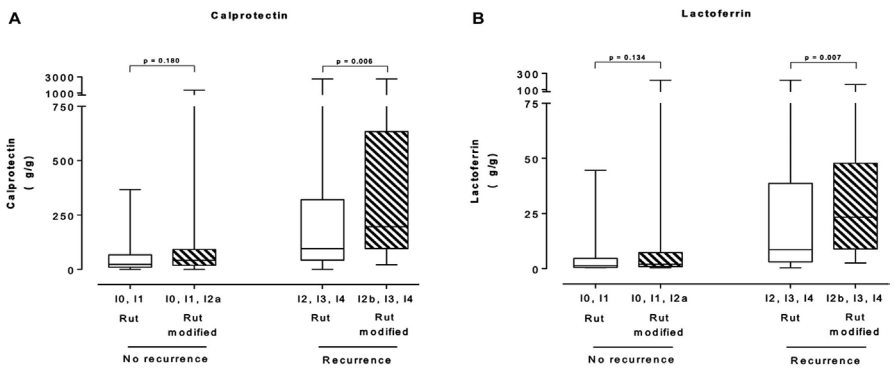

FIGURE 1. FC (A) and FL (B) in endoscopic remission versus recurrence defined by the modified Rutgeerts score and the Rutgeerts score. Median and interquartile range concentrations are presented.

g [IQR 0.55–4.64 μg/g; $P < 0.001$]) (Fig. 1). The 4 patients with upper digestive tract disease had normal values of fecal markers.

A cutoff value of 7.25 μg/g for FL had a sensitivity of 85%, a specificity of 74%, a positive predictive value (PPV) of 64%, a negative predictive value (NPV) of 90%, and an accuracy of 77% in detecting endoscopic recurrence, defined as an MRS ≥ i2b. The calculated best cutoff level for FC in this study for predicting recurrence was 100 μg/g, with a sensitivity of 74%, a specificity of 75%, a PPV of 61%, an NPV of 91%, and an accuracy of 75%. Table 4 shows the sensitivity, specificity, PPV, NPV, and accuracy of FC and FL for predicting endoscopic recurrence (using Rutgeerts and modified Rutgeerts scores) at different cutoff levels.

The area under the ROC curve (AUROC) for FC was 0.831 (95% CI, 0.752–0.911; $P < 0.05$) and for FL it was 0.842 (95% CI, 0.763–0.920; $P < 0.05$) using the modified Rutgeerts score. If recurrence was defined using the Rutgeerts score, the AUROC was 0.757 (95% CI, 0.643–0.871; $P < 0.05$) for FC and 0.767 (95% CI, 0.657–0.877; $P < 0.05$) for FL (Figs. 2 and 3 compare the ROC curves for FC [Fig. 2] and FL [Fig. 3] using the modified Rutgeerts score [A] and the Rutgeerts score [B]).

TABLE 4. Sensitivity, Specificity, PPV, NPV and Accuracy of FC and FL in Identifying Endoscopic Recurrence (Using Rutgeerts and Modified Rutgeerts Scores)

	Sens (%)	Specif (%)	PPV (%)	NPV (%)	Accuracy (%)	AUC (CI 95%)
Rutgeerts						
Calprotectin						0.757 (0.643–0.871)
≥50	72	71	89	45	72	
≥100	48	79	88	33	55	
Lactoferrin						0.767 (0.657–0.877)
≥7.25	55	79	89	37	60	
Modified Rutgeerts						
Calprotectin						0.831 (0.752–0.911)
≥50	94	55	52	95	69	
≥100	74	75	61	91	75	
Lactoferrin						0.842 (0.763–0.920)
≥7.25	85	74	64	90	77	

AUC, area under curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

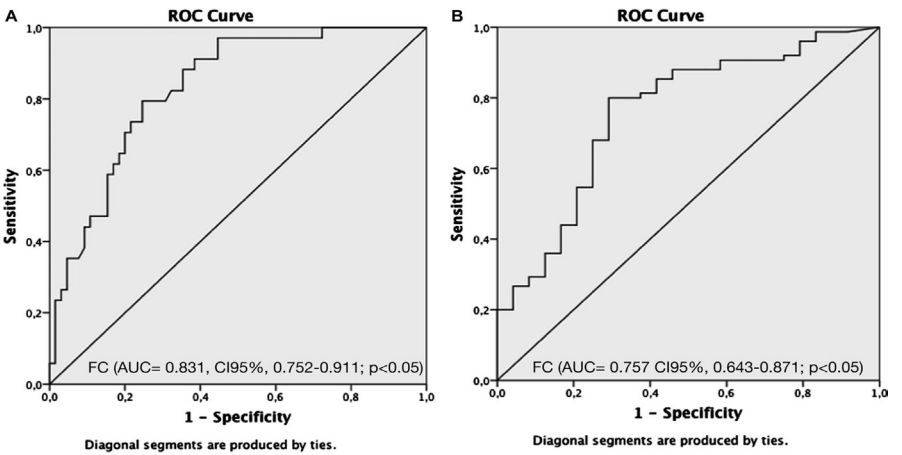

FIGURE 2. ROC curves for FC for discriminating between endoscopic recurrence and remission after surgery (cutoff, 100 μg/g) using modified Rutgeerts score (A) and Rutgeerts score (B).

There was a poor correlation between both fecal markers and HBI (FC, $r_s = 0.096$; FL, $r_s = 0.146$) and also between fecal markers and CRP (FC, $r_s = 0.251$; FL, $r_s = 0.230$) at the time of colonoscopy.

Follow-up

The median follow-up (FU) time since colonoscopy was 25 months (IQR, 22–34). At the end of FU, 95 patients were in clinical remission (HBI < 5). Of the remaining patients, 3 presented with mild and 1 with moderate disease. Of these 4 patients with clinically active disease, only 2 had recurrent endoscopic disease with an MRS of i3 and i4, respectively. No patient needed surgery during the FU period, and 33 patients underwent changes in therapy (10 began immunomodulators, 13 began anti-TNFα agents, 8 switched the anti-TNFα, and 2 began corticosteroids as the only therapy). A course of corticosteroids was added to the therapy in 18 patients. Among the 33 patients that intensified therapy, 20 (60.6%) intensified therapy due to endoscopic recurrence and clinical relapse and the remaining 13 (39.4%) intensified therapy based solely in endoscopic recurrence. We found that patients who needed therapy intensification presented with significantly higher levels of FC and FL (FC, 191.0 μg/g [IQR, 95.6–726.5 μg/g] versus 51.9 μg/g [IQR, 23.4–118.5 μg/g; $P < 0.001$]; FL, 23.5 μg/g [IQR, 8.9–61.9 μg/g] versus 2.5 μg/g [IQR, 1.1–8.4 μg/g]; $P < 0.001$). With regard to endoscopic recurrence, patients with a modified Rutgeerts score ≥ i2b had intensified therapy more often than patients with a modified Rutgeerts score ≤ i2b (71.9% versus 13.8%, $P < 0.001$). Among patients with clinical relapse during follow-up, 16 (80.0%) return to clinical remission at the end of follow-up.

DISCUSSION

There is major scientific evidence to support early endoscopic evaluation of the neoterminal ileum in CD in order to predict the future clinical course and to adjust therapy according to endoscopic findings.[22–24] Recently, the POCER study showed the short-term benefits of postoperative endoscopic evaluation and treatment intensification in recurrent disease.[25] Fecal markers have been attracting great interest in the post-surgery setting despite the conflicting results and the small number of patients enrolled in initial studies,[18,26–29] thereby postponing the establishment of a definitive role of FC and FL in clinical management. In 2013, Yamamoto et al[20] published a pilot study demonstrating that both FC and FL levels correlated with endoscopic scores and higher levels were predictors of clinical recurrence in the 12-month follow-up. In recent years, there have been some new publications supporting the positive value of fecal markers in the evaluation of postoperative recurrence of CD.[25,21,30,31] The study of Wright et al[21] evidenced the predictive value of serial monitoring of FC after surgery for identifying patients likely to relapse and performing early endoscopic evaluation in order to intensify the therapy if recurrence is diagnosed. All published series used the

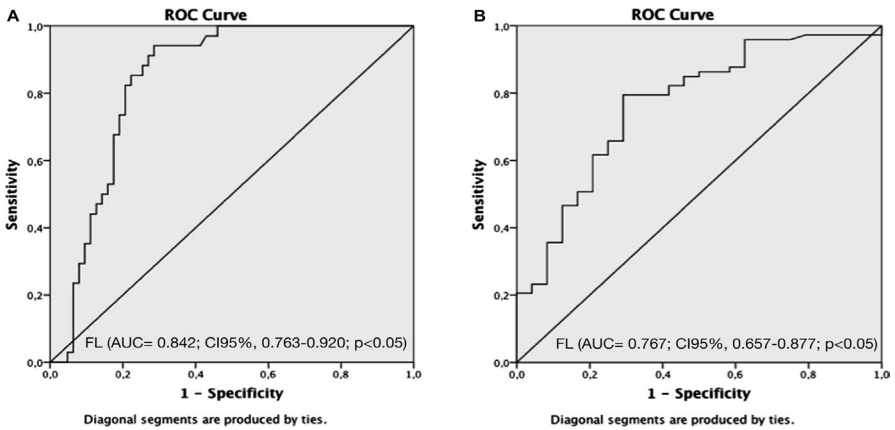

FIGURE 3. ROC curves for FL for discriminating between endoscopic recurrence and remission after surgery (cutoff, 7.25 μg/g) using modified Rutgeerts score (A) and Rutgeerts score (B).

Rutgeerts score to classify endoscopic recurrence, which is still an unvalidated score despite its widespread use in clinical trials and clinical practice. This score lacks interobserver agreement and has been a source of debate as it gathers in the same category patients with lesions in the ileum and patients with lesions confined to the anastomosis. In this study, the authors chose to use the modified Rutgeerts score as, from a theoretical perspective (although again not yet validated), it may be more accurate to define recurrence only when ileal lesions are present (Rutgeerts ≥ i2b). With this score, stenosis and/or ulceration of the anastomosis, which might simply be related to ischemia or staples, do not define recurrent disease and have no prognostic or therapeutic implications. This way, possible confounding factors for recurrent disease would be overcome by the use of this modified score.

This is the first work, to the best of our knowledge, which compares the performance of fecal markers with the 2 scoring systems in predicting recurrent disease. We found that when using the Rutgeerts score, a great number of patients were diagnosed as having recurrent disease. This difference was mainly due to the inclusion of patients with lesions concerning only the anastomosis (either stenosis or ulceration) and no ileal disease (Rutgeerts i2 versus modified Rutgeerts i2a). In addition, the median values of both FC and FL for recurrent disease with the Rutgeerts score were lower than with the modified Rutgeerts score, with the median FC below the cutoff value of 100 μg/g. This probably reflects the subgroup of patients with normal FC and FL that would have been classified as having endoscopic recurrence based only on anastomotic disease. If we had used the Rutgeerts score, the performance of both tests would have been jeopardized, with a decrease in sensitivity from 74% to 48% and in NPV from 91% to 33% with respect to FC, and from 85% to 55% and from 90% to 37%, respectively, for FL. In terms of accuracy, for both tests, we would have a decrease from 75% to 55% for FC and from 77% to 60% for FL. Our findings also suggest, as in other studies, that both FC and FL are sensitive enough to monitor CD recurrence postoperatively, with a high NPV (91% and 90%, respectively), which should reassure clinicians that few patients with endoscopic recurrence will not be diagnosed using these tests. Both markers not only had a good correlation between them but also correlated significantly with the severity of endoscopic findings in the neoterminal ileum.

The presence of active disease elsewhere in the digestive tract, besides the terminal ileum, is one of the major concerns over the use of fecal markers for evaluating postoperative recurrence. In this cohort, no patient presented with active colonic disease and only 5 patients had active disease proximal to the neoterminal ileum, as evaluated by capsule endoscopy, upper endoscopy, or computed tomographic enterography (CTE) (data not shown).

We did not find either an association between CRP levels or clinical symptoms and endoscopic findings, thereby confirming that this variable is not a good predictor of recurrent disease, and that therapeutic options should not only be based on this parameter. In this group, the majority of patients were asymptomatic despite a third of them presenting with recurrent disease.

Selecting the most appropriate cutoff value for FC is critical to its performance as a screening test. Such a value should have a high NPV, so that few patients with active disease would be missed for subsequent colonoscopy. In our analysis, the best cutoff value for FC was 100 μg/g. Compared with 50 μg/g, the value of 100 μg/g had a higher specificity, PPV, and accuracy in diagnosing recurrent disease. If we had used the cutoff of 50 μg/g, we would have increased the sensitivity (74% versus 94%) but with less specificity (75% versus 55%) and accuracy (75% versus 69%). In this study, besides using FC, we decided to evaluate the performance of FL in order to determine whether one marker performed better than the other in diagnosing recurrence, and if we could increase diagnostic accuracy by combining both. One additional factor is the paucity of published data regarding the utility of FL in this context. Although some authors have claimed that FC performs better than FL in the evaluation of IBD,[32] especially in limited ileal CD, the studies published in the literature demonstrate that both markers are useful and comparable[19,20,27] both in differentiating functional from organic disease and in predicting disease activity and relapse.[33–36] Possibly, the limited use of FL relates to a more complex manipulation in the laboratory, bearing in mind the time limitation in the extraction process. FL has also been evaluated in the postsurgery setting in CD, and higher levels correlated with endoscopic recurrence. In our study, FL performed extremely well with sensitivity, specificity, PPV, NPV and accuracy of 85%, 74%, 64%, 90% and 77%, respectively. FC and FL levels paralleled each other and endoscopic findings, but their combined used did not improve diagnostic accuracy (data not shown). Based on these results, it is our conviction that there is no benefit in using both fecal markers in combination because it does not bring any diagnostic improvement.

The major strengths of this study are the following: (1) the number of patients included in a single tertiary centre (99 patients with simultaneous endoscopic assessment, fecal stool, and serum markers evaluated); (2) endoscopic validation by only one experienced operator, which obviates interindividual variation in lesion classification and the definition of recurrence, overcoming one of the major pitfalls when considering endoscopy; (3) the exclusion of upper tract and colonic disease, which may affect FC; and (4) the prospective design. However, we think that the main limitations are the nonhomogeneous and variable time between surgery and endoscopic recurrence evaluation, and the use of a nonvalidated and less known endoscopic score (though the Rutgeerts score has still not been validated either). It is our opinion, supported by these findings, that the modified Rutgeerts score should be preferred over the Rutgeerts score when evaluating postoperative CD. Further studies are needed to validate the modified Rutgeerts score and confirm its better correlation with fecal markers.

In conclusion, fecal markers (FC and LF) are accurate options for selecting patients for endoscopic (re-)evaluation in the postoperative setting. They proved to be superior to clinical scores or serum biomarkers as a screening test for endoscopic recurrence of CD in the postoperative population. Their use should be included in the management algorithm of asymptomatic postsurgery CD patients. Fecal markers performed significantly better when the modified Rutgeerts score was used, suggesting that endoscopic recurrence should only be considered for patients with a modified Rutgeerts score > i2b (see Table, Supplemental Digital Content 1, http://links.lww.com/IBD/B294).

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ORIGINAL ARTICLE

Prospective Study

Fecal marker levels as predictors of need for endoscopic balloon dilation in Crohn's disease patients with anastomotic strictures

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Author contributions: Lopes S and Magro F were responsible for study design; Lopes S and Andrade P were responsible for data collection; Afonso J performed all laboratorial procedures; Rodrigues-Pinto E and Andrade P were responsible for statistical analysis; Lopes S and Andrade P wrote the manuscript; Magro F and Macedo G revised and approved the final manuscript; All the named authors, contributing directly to the work described, have agreed on the respective roles of each author, read the manuscript and approved submission.

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Abstract

AIM

To evaluate the accuracy and best cut-off value of fecal calprotectin (FC) and fecal lactoferrin (FL) to predict disease recurrence in asymptomatic patients presenting with anastomotic strictures.

METHODS

This was a longitudinal single tertiary center study based on prospectively collected data (recorded in a clinical database created for this purpose) performed between March 2010 and November 2014. Crohn's disease (CD) patients with anastomotic stricture who submitted to postoperative endoscopic evaluation were included. Stools were collected on the day before bowel cleaning for FC and FL. Endoscopic balloon dilation (EBD) was performed if the patient presented an anastomotic stricture not traversed by the colonoscope, regardless of patients' symptoms. Successful dilation was defined as passage of the colonoscope through the dilated stricture into the neotermimal ileum.

Postoperative recurrence was defined as a modified Rutgeerts score of ≥ i2b.

RESULTS

In a total of 178 patients who underwent colonoscopy, 58 presented an anastomotic stricture, 86% were asymptomatic, and 48 (54% male; median age of 46.5 years) were successfully dilated. Immediate success rate was 92% and no complications were recorded. FC and FL levels correlated significantly with endoscopic recurrence ($P < 0.001$) with an optimal cut-off value of 90.85 μg/g (sensitivity of 95.5%, specificity of 69.2%, positive predictive value (PPV) of 72.4%, negative predictive value (NPV) of 94.7% and accuracy of 81%] for FC and of 5.6 μg/g (sensitivity of 77.3%, specificity of 69.2%, PPV of 68%, NPV of 78.4% and accuracy of 72.9%) for FL.

CONCLUSION

Fecal markers are good predictors of CD endoscopic recurrence in patients with asymptomatic anastomotic stricture. FC and FL may guide the need for EBD in this context.

Key words: Crohn's disease; Anastomotic strictures; Endoscopic balloon dilation; Fecal markers; Postoperative recurrence

Core tip: This longitudinal study evaluated the accuracy of fecal calprotectin (FC) and fecal lactoferrin (FL) to predict disease recurrence in postoperative Crohn's disease asymptomatic patients with an anastomotic stricture. FC and FL levels accurately predicted endoscopic recurrence in the presence of anastomotic stricture and thus may guide the need for endoscopic balloon dilation (EBD) in this context. A normal value of fecal markers can reassure clinicians and be safely used to avoid balloon dilation if we only aim to diagnose recurrence. A high value of fecal markers has a high likelihood of recurrence so EBD should be performed in order to provide adequate endoscopic therapy and adjust or optimize medical therapy.

Lopes S, Andrade P, Rodrigues-Pinto E, Afonso J, Macedo G, Magro F. Fecal marker levels as predictors of need for endoscopic balloon dilation in Crohn´s disease patients with anastomotic strictures. *World J Gastroenterol* 2017; 23(35): 6482-6490 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i35/6482.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i35.6482

INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory disorder with progression to penetrating or stricturing phenotype as part of the natural history. More than half of CD patients experience these complications during disease lifetime and will need surgery[1,2]. It is well known that disease recurrence proximal or at the anastomosis is almost universal, with progression to luminal narrowing and stricturing behavior[3]. It is known that despite the permanent reduction of luminal caliber and disease progression, the majority of patients remain asymptomatic, with normal biomarkers. As the primary therapeutic goal of CD has shifted from clinical remission to achieving mucosal healing[4,5], it may be important to access the mucosa proximal to strictures to evaluate disease recurrence and escalate therapy if needed. Over the last decade there is increasing evidence for endoscopic balloon dilation (EBD) as a safe and minimally invasive effective method for the treatment of stricturing disease[6-14]. Median technical success has been reported as 90%, with major complication rate around 3%-10%[6,9,10]. Symptomatic recurrence is common, with a reported frequency ranging from 13% to 100%[12]. However, it has been shown that repeated dilations do not reduce the efficacy of the procedure and may prevent surgery in compliant patients[8-12].

Fecal markers, namely fecal calprotectin (FC) and fecal lactoferrin (FL), have proved to be useful and accurate non-invasive tools in evaluating disease activity in CD. Recent works have also demonstrated their validity in diagnosing recurrence in the postoperative setting, suggesting that normal values of fecal biomarkers can obviate the need for endoscopic evaluation[15-18]. In patients with elevated levels of fecal biomarkers, endoscopy should be performed in order to confirm recurrence and escalate therapy if indicated[19]. To our knowledge there are no studies evaluating the performance of FC and FL in patients with asymptomatic anastomotic CD strictures and limited data is available on the long-term effect of medical therapy escalation after balloon dilation of anastomotic strictures.

The aims of this study were, therefore, to evaluate the accuracy of FC and FL in the diagnosis of recurrent disease in the neoterminal ileum in asymptomatic/mild disease patients that had undergone bowel resection for CD and present with stricture of the anastomosis, to evaluate the best cut-off value of FC and FL to diagnose recurrence, and to evaluate the immediate technical success and safety rate of EBD.

MATERIALS AND METHODS

A longitudinal single tertiary center study based on prospectively collected data (recorded in a clinical database created for this purpose), was performed between March 2010 and November 2014. All patients gave informed written consent to participate in the study that was approved by the Ethics Committee of our Institution. From a cohort of consecutive CD

patients who submitted to postoperative endoscopic evaluation after ileocolectomy, we selected the group of patients with anastomotic stricture. All patients were followed at our inflammatory bowel disease (IBD) outpatient clinic and were referred for endoscopic evaluation at our institution.

Inclusion criteria were definitive diagnosis of CD established by standard clinical, radiographic, endoscopic and histological criteria[20], previous ileocolectomy, and existence of an anastomotic stricture. Exclusion criteria were: age less than 18-years-old; strictures length greater than 6 cm; fistulae or deep ulceration of the strictured segment; technical impossibility of passing the catheter/balloon through the strictures; and active disease in the colon or upper digestive tract.

Clinical disease activity was assessed on the day of endoscopic examination, according to the clinical criteria of the Harvey-Bradshaw index (HBI)[21,22]. Clinically inactive disease was defined as HBI < 5. All procedures were performed under propofol sedation, with CO_2 insufflation, by a single senior endoscopist (SL) and on an outpatient basis. Mechanical intestinal bowel preparation was done the day before colonoscopy with polyethylene glycol bowel preparation solution (Klean Prep®; Helsinn Birex Pharmaceuticals, Dublin, Ireland). Stool samples were collected the day before beginning bowel preparation (preferably from the first stool in the morning) and then kept in the refrigerator until being brought to the hospital. For FC, within a maximum 7 d after collection, stools were extracted in accordance with the manufacturer's instructions, using the Fecal Sample Preparation Kit (Roche Diagnostics, Mannheim, Germany) and analyzed using immunoassay (EliA Calprotectin®; Thermo Fisher Scientific, Freiburg, Germany). For FL evaluation, stools were stored at -80 ℃ upon arrival at the laboratory, and samples were thawed and analyzed using a commercially available quantitative enzyme-linked immunoassay test (IBD-Scan®; Tech-Lab, Blacksburg, VA, United States). The techniques for measurement of fecal markers followed the manufacturer´s guidelines.

Postoperative disease activity of the neoterminal ileum was evaluated according to the modified Rutgeerts score[23] (i0: no lesions in the distal ileum; i1: < 5 aphthous lesions in the distal ileum; i2a: lesions confined to the ileocolonic anastomosis, including anastomotic strictures; i2b: > 5 aphthous ulcers or larger lesions, with normal mucosa in between, in the neoterminal ileum, with or without anastomotic lesions; i3: diffuse aphthous ileitis with diffusely inflamed mucosa; i4: large ulcers with diffuse mucosal inflammation or nodules or strictures in the neoterminal ileum). Postoperative recurrence was defined as a modified Rutgeerts score of ⩾ i2b.

EBD was performed if the patient presented an anastomotic stricture not traversed by the colonoscope, regardless of the patient's symptoms. All dilations were performed with the same type of colonoscope (Olympus® CF type H180AL; Tokyo, Japan) under fluoroscopic control to allow the endoscopist to characterize the strictures, exclude peristricture fistulae, evaluate the optimal diameter of the balloon to use and to prompt identify any complication during the procedure. Dilations were performed with a guidewire (Boston Scientific® Jagwire 0.035 in; Marlborough, MA, United States) placed through the strictures [after contrast instillation through a catheter (Olympus® Ball Tip/6Fr) over which a through-the-scope balloon (Cook Medical®, Bloomington, IN, United States) was placed]. The balloon was inflated using contrast agent and the pressure maintained for 2 min, to a maximum diameter of 18 mm.

The procedure could be repeated at the discretion of the endoscopist. Successful dilation was defined as passage of the colonoscope through the dilated stricture into the neotermimal ileum. Only patients with a successful dilation were analyzed, as progression to the neoterminal ileum was mandatory to evaluate disease recurrence. Major complications were defined as major bleeding requiring surgery, blood transfusion or hospital admission and perforation. Minor, self-limited bleeding was not registered as a complication.

Statistical analysis

SPSS 20.0 for Windows (SPSS, Chicago, IL, United States) was used for statistical analysis. Categorical variables were described as absolute frequencies (n) and relative frequencies (%); continuous variables were described as mean ± SD (parametric distributions) or as median and percentiles (non-parametric distributions). The normality of the continuous variables was tested using the Kolmogorov-Smirnov test and the respective histogram. Student's t-test was used to compare quantitative variables with a normal distribution, and the Mann-Whitney U test was used to compare the quantitative variables without a normal distribution. Any groups with more than two quantitative variables were compared using the Kruskal-Wallis test. A Pearson χ^2 test was used to compare categorical variables. Kaplan-Meier analysis with log rank statistics was used to estimate event-free interval. A logistic regression was performed to assess predictors of disease recurrence and need for dilation. Statistical significance was set at $P < 0.05$.

RESULTS

Patient characteristics

One hundred and seventy-eight consecutive CD patients (51.7% male; median age 46.4 years) who previously submitted to right ileocolectomy were evaluated by colonoscopy. At the time of endoscopic evaluation, 31 (17.4%) patients complained of subocclusive symptoms, and 66.3% of patients were being treated with immunomodulators and 38.2% with

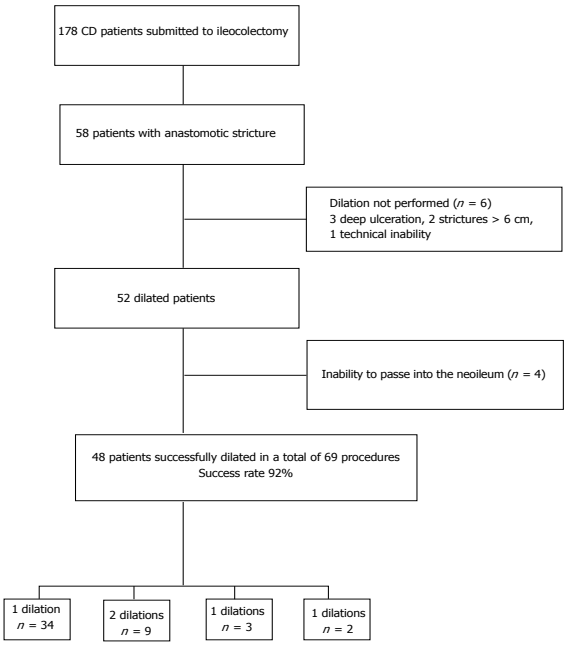

Figure 1 Study flowchart. CD: Crohn's disease.

biologics.

Of the 178 evaluated patients, 58 (32.6%) presented with an anastomotic stricture. The majority were asymptomatic, with only 8 (13.8%) patients presenting with subocclusive symptoms. All patients were in clinical remission (HBI < 5 in 83.3%) or with mild clinical disease (HBI 5-7 in 16.7%). Among the 58 patients presenting with anastomotic stricture, 52 were dilated (6 were excluded due to deep ulceration, stricture size > 6 cm or technical inability). Of the total 52 dilated patients, 4 were excluded as it was not possible to evaluate the neoterminal ileum (Figure 1). Baseline characteristics of the 48 successfully dilated patients are summarized in Table 1. The majority were men (54%) with a median age of 46.5 years. At the time of dilation, 42% of patients were being treated with biologics and 54% with immunomodulators.

EBD

Overall, technical success rate was 92% (48/52) and no major complications were recorded. The 18-mm balloon diameter was used in 69.2% of the patients and the 15-mm diameter in 25.0%. Redilation was required in 14 patients (29.2%) during a median follow-up time of 34 mo (27.5-52.5 mo). During the follow-up period, only 2 patients needed surgery, both cases due to long strictures that did not allow EBD (Table 2).

Fecal markers and endoscopic recurrence

Of the 48 successfully dilated patients, 22 presented with endoscopic recurrence defined as modified Rutgeerts score of ⩾ i2b. Of these, 16 patients presented with severe disease (i3 = 3 and i4 = 13). Recurrence was diagnosed only after dilation of the anastomotic stricture and intubation of the neoterminal ileum. Comparing FC and FL levels in patients with endoscopic recurrence and in patients with endoscopic remission we found a significantly higher level in patients with endoscopic recurrence [FC: 257.0 μg/g, interquartile range (IQR): 161.0-565.0 μg/g) *vs* 53.9 μg/g, IQR: 23.9-146.0 μg/g; $P < 0.001$ and FL: 9.1

Table 1 Patient characteristics, *n* = 48

Characterization	*n* (%)
Women,	22 (45.8)
Median time between diagnosis and surgery, mo (IQR)	36.0 (14.0-120.0)
Median time between surgery and endoscopic evaluation, mo (IQR)	114.5 (60.8-199.0)
Median age at endoscopic evaluation, yr (IQR)	46.5 (39.3-53.4)
Montreal classification	
Age at diagnosis	
A1, ≤ 16 yr	6 (12.5)
A2, 17-40 yr	33 (68.8)
A3, > 40 yr	9 (18.8)
Location	
L1: ileal	30 (62.5)
L3: ileocolonic	17 (35.4)
L4: upper gastrointestinal tract	1 (2.1)
Behavior	
B1: non-stricturing, non-penetrating	1 (2.1)
B2: stricturing	27 (56.2)
B3: penetrating	20 (41.7)
Perianal disease	14 (29.2)
Smoking	
Never	26 (54.2)
Current	12 (25.0)
Past	10 (20.8)
Concomitant treatment	
Corticosteroids	9 (18.8)
Immunomodulators (Azathioprine/6MP/ Methotrexate)	26 (54.2)
Biologics (Infliximab, adalimumab)	20 (41.7)
5-ASA	17 (35.4)
Median fecal calprotectin, μg/g (IQR)	134.0 (35.3-321.0)
Median, fecal lactoferrin, μg/g (IQR)	6.2 (2.0-22.4)
Modified Rutgeerts score	
i0, i1, i2a	26 (54.2)
i2b, i3, i4	22 (45.8)
Subocclusive symptoms	8 (16.7)
Harvey-Bradshaw index	
Remission (HBI < 5)	40 (83.3)
Mild disease (HBI 5-7)	8 (16.7)
Need of redilation	14 (29.2)
Surgery after dilation	2 (1.4)
Median follow up, mo (IQR)	34.0 (27.5-52.5)

HBI: Harvey-Bradshaw index; IQR: Interquartile range.

μg/g, IQR: 5.5-27.8 μg/g *vs* 3.9 μg/g, IQR: 1.5-21.9 μg/g; *P* = 0.042] (Figure 2). No other clinical variable or biomarker reached statistical difference between the two groups (Table 3).

The best calculated cut-off value for FC to predict recurrent disease was 90.85 μg/g, with a sensitivity of 95.5%, a specificity of 69.2%, a positive predictive value (PPV) of 72.4%, a negative predictive value (NPV) of 94.7% and an accuracy of 81%. The area under the ROC curve for diagnosing endoscopic recurrence was 0.786 (95%CI: 0.646-0.926, *P* < 0.05) for FC. Concerning FL, the best calculated cut-off was 5.6 μg/g, with sensitivity of 77.3%, specificity of 69.2%, PPV of 68%, NPV of 78.4% and accuracy of 72.9%. The area under the ROC curve for diagnosing endoscopic recurrence was 0.672 (95%CI: 0.511-0.834, *P* = 0.042) (Figure 3).

Table 2 Characterization of the dilation procedure

Characterization	*n* (%)
Patients with stricture	58 (32.6)
Stricture type	
Anastomotic	58 (100)
Dilated patients	52 (29.2)
Causes for non-dilation	
Length of stenosis	2 (33.3)
Ulceration of mucosa	3 (50.0)
Technical inability	1 (16.7)
Successful dilated patients	
Balloon size, *n* = 52	48 (92.3)
15 mm	13 (25.0)
16.5 mm	3 (5.8)
18 mm	36 (69.2)
Complications	0 (0.0)

DISCUSSION

One of the major drawbacks of CD surgical therapy is the high recurrence rate of the disease at the anastomotic site or in the neoterminal ileum, with the development of stricture and bowel obstruction[8]. As the primary therapeutic goal of CD has shifted from clinical remission to achieving mucosal healing[4,5], it may be important to access the mucosa proximal to strictures to evaluate disease recurrence and escalate therapy if needed. The recently published POCER trial[16] demonstrated that initial postoperative treatment according to clinical risk stratification and early endoscopic evaluation and step-up therapy if there was recurrent disease was superior to standard medical therapy.

In this study, endoscopy was the gold standard method to define recurrent disease. With respect to non-invasive methods, both serum biomarkers and clinical activity indexes have demonstrated a poor correlation with disease activity[24-26]. The use of fecal markers in the postoperative period has been studied in small groups of patients with variable results. Recently, however, some studies evaluated the value of fecal biomarker measurement after surgery for CD. In 2015, Wright *et al*[15] demonstrated that FC was sensitive enough to diagnosis CD recurrence in 135 patients submitted to bowel resection with a high enough NPV to reassure clinicians that few patients with recurrence would be missed. More recently, Lopes *et al*[27] showed that both FC and FL strongly correlated with endoscopic findings in the evaluation of CD after surgery and accurately predicted endoscopic recurrence in 99 CD patients who submitted to ileocolonic resection.

The results of both of these studies suggests that fecal biomarkers may be incorporated in the postoperative management algorithm, both to diagnose recurrence and to assess response to therapy. In our study, we evaluated if in asymptomatic CD patients with anastomotic strictures not traversed

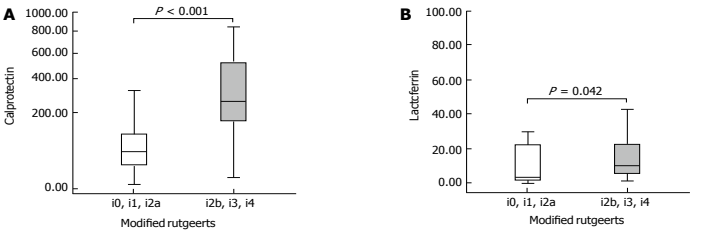

Figure 2 Box plots of fecal markers according to endoscopic activity defined by the modified Rutgeerts score, in asymptomatic patients presenting with anastomotic strictures. A: Fecal calprotectin; B: Fecal lactoferrin.

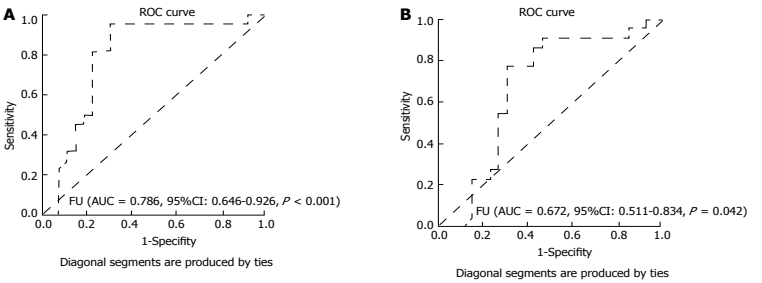

Figure 3 Receiver operating characteristic curves for fecal markers for discriminating between endoscopic recurrence and remission in asymptomatic patients presenting with anastomotic strictures. A: Fecal calprotectin; B: Fecal lactoferrin.

by the colonoscope, fecal markers perform as well as a predictor of disease recurrence, defining groups of patients that will need more invasive methods. In this series of 48 patients with strictures, only 18% complained of subocclusive symptoms. Similarly to what has been published[28-30], we also did not find any correlation between patient symptoms, serum biomarkers and HBI and endoscopic or radiographic findings, supporting the belief that using only symptoms or C-reactive protein levels to inform treatment decisions may increase the risk of disease progression and complications.

It is controversial whether or not asymptomatic strictures should be endoscopically treated. Despite being a safe and minimally invasive technique, there is a 3%-10%[6,9,10] described risk of major complications, especially in centers with limited procedural volume per year. Our data confirmed the safety and efficacy of EBD in the context of CD anastomotic strictures, with a technical success rate of 92%. We had no serious complications, which may be explained by several factors: careful patient selection; use of fluoroscopic image to evaluate, in real time, stricture characteristics and the therapeutic procedure indicated; maximum diameter of the balloon used (18 mm); use of carbon dioxide as type of insufflation; and experience of the endoscopist performing the technique with a uniform technical approach. The dilation of the anastomosis allowed the diagnosis of recurrence in 22 patients, that otherwise would have been missed if we only relied on symptoms or biochemical markers.

We used the modified Rutgeerts score to diagnose endoscopic recurrence, although it is not yet validated. Despite being used for several decades in clinical trials and clinical practice, the Rutgeerts score is also an invalidated score. In this study, we chose to use the modified Rutgeerts score, as demonstrated in a previous work[27] as having a better correlation between the modified score and fecal markers to diagnose recurrent disease. Recurrence was defined by a modified Rutgeerts score of i2b, not considering the presence of only anastomotic stricture as a criterion, as many other factors may be implicated in stricture development[1,2]. In that paper[27] the calculated best cut-off level for FC for predicting recurrence was 100 μg/g, with a sensitivity of 74% and a NPV of 91%.

Table 3 Comparison between patients with and without endoscopic recurrence

	Endoscopic recurrence, *n* = 22	No endoscopic recurrence, *n* = 26	*P* value
Sex, M:F	11:11	15:11	0.404
Median age, yr (IQR)	47.0 (35.5-53.3)	46.5 (39.8-54.8)	0.472
Median hemoglobin, g/dL (IQR)	13.8 (12.5-15.1)	13.9 (13.1-15.0)	0.715
Median albumin, g/L (IQR)	42.4 (36.1-45.9)	42.1 (39.8-44.6)	0.886
Median C-reactive protein, mg/L (IQR)	5.6 (1.6-8.2)	2.4 (0.9-9.9)	0.457
Median fecal calprotectin, μg/g (IQR)	257.0 (161.0-565.0)	53.9 (23.9-146.0)	< 0.001
Median fecal lactoferrin, μg/g (IQR)	9.1 (5.5-27.8)	3.9 (1.5-21.9)	0.042
Smoking, yes/no/past	3/13/6	9/13/4	0.196
Subocclusive symptoms, yes/no	2/20	6/20	0.183
HBI, remission/mild	17/5	24/2	0.145
Concomitant treatment			
Anti-TNF- α agents, yes/no	12/10	14/12	0.596
Immunomodulators, yes/no	9/13	11/15	0.578
Steroids, yes/no	4/18	5/21	0.611

HBI: Harvey-Bradshaw index; IQR: Interquartile range.

This is in accordance with other studies[15] that defined in the post-operative setting a higher cut-off than that of 50 μg/g used to diagnose inflammatory bowel disease[31].

In the present study, both FC and FL were also significantly higher in patients with endoscopic recurrence, with an area under the receiver operating characteristic curve for detection of endoscopic recurrence of 0.786 for FC and of 0.672 for FL. The best cut-off value of FC as predictor of recurrence was 90.85 μg/g, with sensitivity of 95.5%, NPV of 94.7% and accuracy of 81%. If we adopted the commonly used cut-off value of 7.25 μg/g for lactoferrin[32-34], we would have missed patients with recurrence (false negative results). The cut-off value of 5.6 μg/g had sensitivity of 77.3%, NPV of 78.4% and accuracy of 72.9%. Our findings support the potential value of these two noninvasive markers in the monitoring of patients submitted to bowel resection and presenting with an anastomotic stricture.

Indeed, a FC and/or FL concentration lower than 90.85 μg/g and 5.6 μg/g respectively, have high accuracy to exclude disease recurrence, with no need to further therapeutic intervention. This may be of particular interest in centers with low expertise in EBD and/or with a lower procedural volume per year, in order to avoid complications and/or be used as an indication for referring patients to tertiary centers. If these results are reproduced and validated by others, in a large number of patients, this conservative strategy could be adopted, reserving balloon dilation for symptomatic patients and those with high levels of fecal markers, in order to facilitate step-up therapy.

To our knowledge this is the first report on the performance of FC and FL in the context of anastomotic strictures in CD. Our results suggest that low values of fecal markers can predict, with a great amount of certainty, disease remission. This may avoid application of endoscopic dilation in asymptomatic patients in centers with less endoscopic expertise, reassuring physicians that the use of fecal markers serves as a good indicator to monitor disease recurrence. The serial monitoring of FC and FL can help to make decisions in indeterminate results and a persistently elevated value may serve as another useful indicator when considering therapy intensification.

In conclusion, postoperative FC and FL levels accurately predicted endoscopic recurrence in the presence of anastomotic stricture. Considering that a significant number of patients remain asymptomatic, with normal serum biomarkers, despite the permanent reduction of luminal caliber, a normal value of fecal markers can reassure clinicians and be safely used to avoid balloon dilation if we only aim to diagnose recurrence. In asymptomatic patients with a high FC and/or FL level, there is a great chance of having disease activity proximal to the stricture, so EBD should be performed in order to provide adequate endoscopic therapy and adjust or optimize medical therapy.

COMMENTS

Background

Recurrent disease in the neoterminal ileum and anastomotic strictures are frequent complications of Crohn´s disease (CD). Despite the permanent reduction of luminal caliber and disease progression, the majority of patients remain asymptomatic. Fecal calprotectin (FC) and lactoferrin have been suggested as surrogate non-invasive markers for diagnosing postoperative disease recurrence. There are no studies evaluating the performance of fecal markers as predictors of disease recurrence in asymptomatic patients with an anastomotic stricture.

Research frontiers

The results demonstrated that FC and lactoferrin are good predictors of CD endoscopic recurrence in patients with asymptomatic anastomotic stricture and may guide the need for endoscopic balloon dilation in this context.

Innovations and breakthroughs

A normal value of fecal markers can reassure clinicians and be safely used to avoid balloon dilation if we only aim to diagnose recurrence. A high value of fecal markers has a high likelihood of recurrence, so endoscopic balloon dilation should be performed in order to provide adequate endoscopic therapy and adjust or optimize medical therapy.

Applications

Fecal markers may avoid the need of endoscopic balloon dilation in asymptomatic patients with anastomotic stricture if we only aim to diagnose disease recurrence.

Terminology

Calprotectin is a protein complex, constituting up to 60% of neutrophil cytosol

protein that is released upon neutrophil activation. Lactoferrin, an iron-binding protein, is the main component of secondary granules that degranulate during inflammatory process. Both these proteins are remarkably stable and resistant to degradation, easily detected and have been proved to reflect endoscopic disease activity in CD, predicting endoscopic inflammation and being a surrogate marker of mucosal healing.

Peer-review

The authors have performed a very interesting and important study. They concluded that postoperative FC and FL levels accurately predicted endoscopic recurrence in the presence of anastomotic stricture.

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ORIGINAL ARTICLE

Retrospective Study

Endoscopic balloon dilation of Crohn's disease strictures-safety, efficacy and clinical impact

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Author contributions: Lopes S and Rodrigues-Pinto E contributed equally in the design, conception, analysis, and paper writing; Lopes S, Rodrigues-Pinto E and Magro F conceived and designed the study; Lopes S, Rodrigues-Pinto E and Andrade P collected and analyzed the data; Afonso J performed all laboratorial procedures; Rodrigues-Pinto E and Andrade P were responsible for statistical analysis; Baron TH, Magro F and Macedo G participated in critical revision of the manuscript.

Institutional review board statement: The study was approved by the Ethics Committee of Centro Hospitalar São João, Porto, Portugal.

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Abstract

AIM

To evaluate the incidence of anastomotic strictures after intestinal resection in Crohn's disease (CD), demonstrate long-term efficacy and safety of endoscopic balloon dilation (EBD) in CD strictures and its impact on the diagnosis of subclinical postoperative endoscopic recurrence.

METHODS

Retrospective single tertiary center study based on prospectively collected data between 2010 and 2015

including anastomotic and non-anastomotic strictures.

RESULTS

29% of 162 CD patients included developed an anastomotic stricture. 43 patients with anastomotic strictures and 37 with non-anastomotic strictures underwent EBD; technical success was 97.7% and 100%, respectively, however, 63% and 41% needed repeat dilation during the 4.4-year follow-up. Longer periods between surgery and index colonoscopy and higher lactoferrin levels were associated with the presence of stricture after surgery. Calprotectin levels > 83.35 μg/g and current or past history of smoking were associated with a shorter time until need for dilation (HR = 3.877, 95%CI: 1.480-10.152 and HR = 3.041, 95%CI: 1.213-7.627). Anastomotic strictures had a greater need for repeat dilation (63% *vs* 41%, *P* = 0.047). No differences were found between asymptomatic and symptomatic cohorts. Disease recurrence diagnosis was only possible after EBD in a third of patients.

CONCLUSION

EBD is an effective and safe alternative to surgery, with a good short and long-term outcome, postponing or even avoiding further surgery. EBD may allow to diagnose disease recurrence in patients with no clinical signs/biomarkers of disease activity.

Key words: Crohn's disease; Endoscopic recurrence; Anastomotic strictures; Non-anastomotic strictures; Endoscopic balloon dilation

Core tip: This study evaluated the incidence of anastomotic strictures after intestinal resection in Crohn's disease (CD), the long-term efficacy and safety of endoscopic balloon dilation (EBD) in CD strictures and its impact on the diagnosis of subclinical postoperative endoscopic recurrence. Almost one-third of CD patients developed an anastomotic stricture after ileocecal resection/right hemicolectomy. EBD was an effective and safe alternative to surgery, with a good short and long-term outcome, postponing or even avoiding further surgery. EBD also allowed to diagnose disease recurrence in patients with no clinical signs/biomarkers of disease activity. Longer intervals after surgery and higher lactoferrin levels were associated with anastomotic strictures; time until dilation was lower in patients with calprotectin levels > 83.35 μg/g and current/past history of smoking.

Lopes S, Rodrigues-Pinto E, Andrade P, Afonso J, Baron TH, Magro F, Macedo G. Endoscopic balloon dilation of Crohn's disease strictures-safety, efficacy and clinical impact. *World J Gastroenterol* 2017; 23(41): 7397-7406 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i41/7397.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i41.7397

INTRODUCTION

Strictures in Crohn's disease (CD) develop during the course of the disease or as the presenting feature[1]. Up to 50% of CD patients undergo surgical resection within the first 10 years of diagnosis[2]. Disease recurrence often occurs at or above the anastomosis due to ongoing inflammatory activity[3,4]. This can result in luminal narrowing, and strictures (non-anastomotic and anastomotic), with up to 70% of patients requiring additional resection[5], though is unpredictable[6].

Medical therapy for stricture management is limited due to the fibrotic nature. Management includes surgical resection and stricturoplasty but with a high rate of recurrence and need for reoperation[7]. Increasing evidence supports endoscopic balloon dilation (EBD) as a safe and effective alternative to surgery, particularly for ileocecal and anastomotic strictures[8,9]. Technical and clinical success rates (resolution of obstructive symptoms) are seen in 73%-100% and 64%-70%, respectively, with a major adverse event (AE) rate of 2%-6.4%[1,10-12]. Balloon diameters of 25 mm are believed to increase risk of AEs[13]. During long-term follow-up patients needing surgery at 1, 3 and 5 years varies form 13%-17%, 28%-42%, and 36%-42% respectively. Strictures recur following dilation, and re-dilation may be required in up to 20% and 50% by 1 and 5 years, respectively[1,14,15]. The best results following dilation are obtained when stricture length is < 4 cm, and for anastomotic strictures when compared to *de novo* strictures[1,12,16].

Anastomotic strictures may represent disease recurrence, but data is limited and contradictory as to whether escalation of medical therapy following dilation may prevent the need for repeat dilation or surgery[10,12]. Other factors such as smoking status and disease activity status at the time of dilation may affect outcome of stricture dilation[12], though many studies are limited by short follow-up durations and small cohorts.

We sought to evaluate anastomotic stricture development after intestinal resection in CD and demonstrate long-term efficacy and safety of EBD in CD anastomotic and *de novo* strictures in a large referral centre cohort and determine the impact of dilation on the diagnosis of subclinical postoperative endoscopic recurrence.

MATERIALS AND METHODS

Retrospective single tertiary center study based on prospectively collected data from a clinical database

created for this purpose. Patients were treated from March 2010 to February 2015 including CD patients who had undergone ileocecal resection/right hemicolectomy. All patients were followed at our Inflammatory Bowel Disease (IBD) outpatient clinic and referred for endoscopic evaluation. Inclusion criteria were definitive diagnosis of CD established by clinical, radiographic, endoscopic, and histological criteria and previous surgery and surgical pathology. Exclusion criteria were previous EBD, age < 18 years, stricture length > 6 cm and fistulae or deep ulceration of the strictured segment.

Clinical disease activity was assessed on the day of endoscopic examination, using the Harvey Bradshaw Index (HBI). Clinically inactive disease was defined as a HBI < 5. Postoperative disease activity of the neoterminal ileum was evaluated according to the Modified Rutgeerts' score[17]. Indication for EBD was to evaluate endoscopic recurrence or symptom/biomarker-driven.

CD patients with non-anastomotic strictures who underwent EBD during the study period were included as a control group (Figure 1).

All procedures were performed in an outpatient setting under propofol sedation, with CO_2 insufflation, by one of two endoscopists (SL and ERP). Polyethylene glycol based bowel preparation was administered the day before colonoscopy. EBD was performed for strictures that would not allow passage with a colonoscope, regardless of patients' symptoms. Dilations were performed endoscopically with a through-the-scope balloon (Boston Scientific, Marlborough, MA), of 10-18 mm diameter and lengths of 55 mm. The balloon was filled with diluted contrast, with diameter of the balloon chosen according to endoscopist discretion. Inflation pressure was maintained for 2 min.

Technical success was defined as the ability to pass the colonoscope through the stricture into the neoileum following dilation. Clinical success was defined as improvement of obstructive symptoms (in symptomatic patients). Major AEs were defined as major bleeding (requiring surgery, blood transfusion or hospital admission) and perforation. Minor, self-limited bleeding was not considered an AE. All patients who underwent dilation were endoscopically reevaluated 6-12 mo later. Long-term efficacy was defined as avoidance of surgical resection or repeat dilation after the initial dilation. Patients were followed until stricture resection, last clinic follow-up, or censor date of March, 2017. Escalation of medical therapy was defined as initiation of a thiopurine or anti-TNF within 6 mo of first dilation, as determined by global physician assessment.

All patients gave informed written consent to participate in the study that was approved by the Ethics Committee of our Institution.

Statistical analysis

Categorical variables were described through absolute and relative frequencies. Continuous variables were described as median, minimum and maximum and dichotomized for analysis using the best cut-off on ROC analysis. Hypotheses were tested about the distribution of continuous variables with non-normal distribution, by using the nonparametric Mann-Whitney. The Chi-squared test and Fisher's exact test were used for differences in proportions of patients experiencing a given outcome. Univariate and multivariate analysis by logistic regression was used to explore the correlation between predictor variables and need of dilation after surgery, as well as need to repeat dilation. To identify independent predictors of need of dilation after surgery, as well as need to repeat dilation, all significant variables evaluated in the univariate analysis were included. Kaplan-Meier survival analysis with log rank statistics was used to assess event-free survival, and Cox conditional proportional hazards regression analysis was used to time-free survival. The results are shown as odds ratio (OR) and hazards ratio (HR) with 95% confidence intervals (CI). All the reported *P* values were two-sided, and *P* values of < 0.05 were considered statistically significant. All data were arranged, processed and analyzed with SPSS® v.24.0 data (Statistical Package for Social Sciences).

RESULTS

Population

A total a 162 CD patients (52.5% males, *n* = 85) who had undergone ileocecal resection/right hemicolectomy were included; the mean age was 42.6 years (SD ± 13.4 years). Baseline demographic characteristics are listed in Table 1. The median follow-up period since colonoscopy index was 4.4 years (1.3-6.8), with a median disease duration of 17.1 years (3.3-52.1). Median time between surgery and index colonoscopy was 7.7 years (range 0.3-37.6 years).

At the time of index colonoscopy, 82% of patients (*n* = 133) were receiving CD medication: 68.5% (*n* = 111) thiopurines and 36.4% (*n* = 59) anti-TNF medication; only 23% of the patients (*n* = 37) were on combination therapy. Seventeen percent of the patients (*n* = 27) had obstructive symptoms, with a median HBI of 1 (0-9). Median labs were: haemoglobin 13.5 g/dL (9.6-18.3), albumin 42 g/dL (24.2-352), C-reactive protein 2.8 mg/L (0.1-105.3), median lactoferrin 4.7 μg/g (0.4-216) and median calprotectin 68.5 μg/g (0.5-2051).

Anastomotic strictures

Twenty-nine percent of patients (*n* = 47) had an anastomotic stricture (17 symptomatic), with 4 also having a non-anastomotic stricture; dilation wasn't

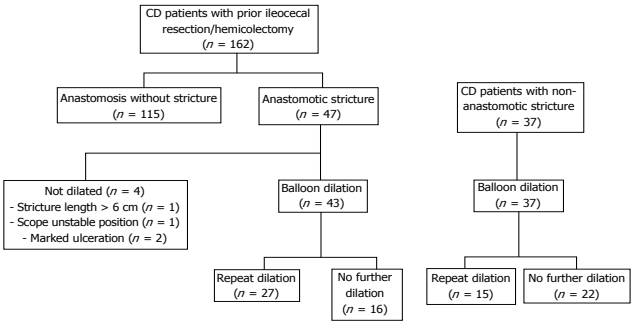

Figure 1 Flowchart with study design.

Table 1 Baseline characteristics of crohn's disease patients with prior ileocecal resection/right hemicolectomy

Characteristic	CD (*n* = 162)
Female/male (*n*)	77/85
Disease duration in years (median; min-max)	17.1 (3.3-52.1)
Follow-up time in years (median; min-max)	4.4 (1.3-6.8)
Time between surgery and index colonoscopy in years (median; min-max)	7.7 (0.3-37.6)
Age at index colonoscopy (mean; standard deviation)	42.6 (± 13.4)
Montreal classification (*n*; %)	
Age at diagnosis	
A1	25 (15.5)
A2	116 (72)
A3	20 (12.4)
Disease location	
L1	89 (54.9)
L2	9 (5.6)
L3	55 (34)
L1-4	7 (4.3)
L3-4	2 (1.2)
Behavior	
B1	6 (3.7)
B2	77 (47.5)
B3	79 (48.8)
Perianal disease	38 (23.5)
Smoking habits (*n*; %)	
Non-smoker	86 (55.1)
Ex-smoker	34 (21.8)
Current smoker	36 (23.1)
Medication at index colonoscopy	
Thiopurines	111 (68.5)
Anti-TNFα	59 (36.4)

TNFα: Tumor necrosis factor α.

performed in 4 patients: 1 due to stricture length > 6 cm, 2 due to marked ulceration and 1 due to unstable scope position.

The presence of stricture after surgery was associated with presence of obstructive symptoms (37% *vs* 9%, OR = 6.3, 95%CI: 2.5-14.9, $P < 0.001$), no medication with thiopurines (74.8% *vs* 53.2%, OR = 2.6, 95%CI: 1.3-5.3, $P = 0.007$), longer duration between surgery and index colonoscopy [132 mo (9-439) *vs* 71 mo (3-415), $P = 0.001$], older age [47 years (16-72) *vs* 39 years (18-77), $P = 0.031$] and lower C-reactive protein levels [2.65 mg/L (0.3-21.9) *vs* 2.85 mg/L (0.1-105.3), $P = 0.045$].

On univariate analysis, the presence of stricture after surgery was associated with longer periods between surgery and index colonoscopy (OR = 1.006, 95%CI: 1.003-1.010, $P = 0.001$), higher lactoferrin levels (OR = 1.010, 95%CI: 1.000-1.021, $P = 0.049$), obstructive symptoms (OR = 6.155, 95%CI: 2.546-14.880, $P < 0.001$), no medication with thiopurines (OR = 2.611, 95%CI: 1.282-5.319, $P = 0.008$) and older age at *index* colonoscopy (OR = 1.028, 95%CI: 1.002-1.055, $P = 0.033$) (Table 2). On multivariate analysis, only longer periods between surgery and index colonoscopy (OR = 1.007, 95%CI: 1.001-1.013, $P = 0.027$) and higher lactoferrin levels (OR = 1.012, 95%CI: 1.000-1.024, $P = 0.043$) were associated with the presence of stricture (Table 2).

In the Cox regression univariate analysis, time until dilation was longer in patients on medication with thiopurines or anti-TNF (HR = 0.476, 95%CI: 0.239-0.946, $P = 0.034$); in further subgroup analysis, thiopurine treatment was the only medication found to be significantly associated with a longer time to dilation (HR = 0.493, 95%CI: 0.275-0.883, $P = 0.017$). Obstructive symptoms and calprotectin levels higher than 83.35 μg/g, on the other hand, were associated with a shorter time until dilation (HR = 2.976, 95%CI: 1.622-5.460, $P < 0.001$ and HR = 3.444, 95%CI: 1.391-8.526, $P = 0.008$, respectively). There was a trend for current or past history of smoking being associated with a shorter time to dilation in the univariate analysis (HR = 1.752, 95%CI: 0.964-3.185, $P = 0.066$). In the Cox multivariate analysis, calprotectin levels > 83.35 μg/g and current

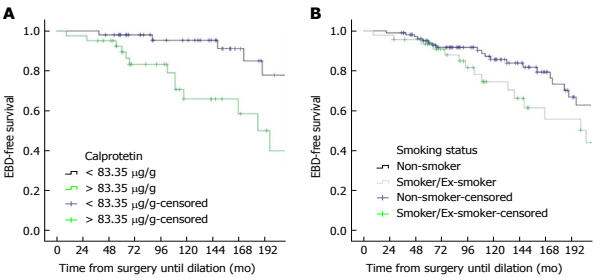

Figure 2 Kaplan-Meier curves showing time from surgery to dilation (in mo) considering calprotectin levels (A) and smoking status (B).

Table 2 Univariate and multivariate analysis of risk factors for need for dilation after surgery

Risk factors	Univariate analysis			Multivariate analysis		
	OR	95%CI	*P* value	OR	95%CI	*P* value
Significant in univariate and multivariate analysis						
Time between surgery and index colonoscopy	1.006	1.003-1.010	0.001	1.007	1.001-1.013	0.027
Lactoferrin levels	1.010	1.000-1.021	0.049	1.012	1.000-1.024	0.043
Significant in univariate analysis						
Subocclusive symptoms	6.155	2.546-14.880	< 0.001	-	-	0.180
No medical treatment with thiopurines	2.611	1.282-5.319	0.008	-	-	0.500
Age at index colonoscopy	1.028	1.002-1.055	0.033	-	-	0.932
Not significant in univariate nor multivariate analysis						
Smoking status	1.877	0.912-3.861	0.087			
B2 behavior	1.221	0.619-2.408	0.565			
B3 behavior	0.895	0.454-1.767	0.750			
Perianal disease	1.376	0.632-2.996	0.421			
Anti-TNF therapy	0.893	0.443-1.799	0.751			
C-reactive protein levels	0.975	0.935-1.016	0.229			
Calprotectin levels	1.001	1.000-1.002	0.125			
Harvey-Bradshaw index	1.111	0.936-1.318	0.229			
Disease duration	1.002	1.000-1.005	0.089			

OR: Odds ratio; CI: Confidence interval; B1: Non-stenosing and non-penetrating behaviour; B2: Stenosing behaviour; B1: Penetrating behaviour; TNF: Tumor necrosis factor.

or past history of smoking were associated with a shorter duration to dilation (HR = 3.877, 95%CI: 1.480-10.152, *P* = 0.006 and HR = 3.041, 95%CI: 1.213-7.627, *P* = 0.018) (Figure 2).

Endoscopic balloon dilation

EBD was performed in 26.5% (*n* = 43) of patients (Table 3). Technical success was achieved in 97.7% (*n* = 42). Repeat dilation was required in 62.8% (*n* = 27) of patients (long-term efficacy: 37.2%), with a total of 85 dilations being performed during the study period [median: 2 (1-5)], with a median balloon dilation of 18mm (range 10-18). Technical success was achieved in 95.3% (*n* = 81) without major AEs, and only one episode of self-limited bleeding (1.2%). All patients (*n* = 17) had improvement of obstructive symptoms. Median time to second dilation was 453.5 d (range: 152-1362).

Endoscopic recurrence, defined as modified Rutgeerts score ≥ i2b was present in 60 patients (37.7%), of which 20 (33%) were diagnosed only after dilation of the anastomotic stricture. Following initial endoscopy and dilation, medical therapy was escalated in 38.5% of patients (*n* = 15; 3 began thiopurines and 14 began anti-TNF); however, escalation of medical therapy was probably driven by endoscopic recurrence (87%, *n* = 13) and not by anastomotic stricture presence, as suggested by escalation of therapy in 53% (*n* = 21) of the patients with endoscopic recurrence and no anastomotic stricture. No agreement was found between endoscopic recurrence and presence of stricture (*K* = 0.085, *P* = 0.273). Escalation of medical therapy did not decrease the need for repeat dilation; no other risk factors (gender, age, Montreal classification, perianal disease, smoking habits, previous medical therapy, presence of obstructive

Table 3 Baseline characteristics comparison between anastomotic and non-anastomotic strictures *n* (%)

Characteristic	Anastomotic strictures (*n* = 43)	Non-anastomotic strictures (*n* = 37)	*P* value
Female/male (*n*)	19/24	22/15	0.173
Disease duration in years (median; min-max)	19.2 (5.7-52.1)	16.3 (3.0-45.3)	0.236
Follow-up since 1st dilation in years (median; min-max)	4.4 (1.3-6.8)	2.9 (1.2-6.5)	< 0.001
Time between 1st and 2nd dilation in days (median; min-max)	453.5 (152-1362)	368 (157-1705)	0.796
Age at index colonoscopy (mean; standard deviation)	44.9 (± 12.2)	39 (± 12.2)	0.035
Montreal classification			
Age at diagnosis			0.368
A1	6 (14)	4 (12.9)	
A2	29 (67.4)	25 (80.6)	
A3	8 (18.6)	2 (6.5)	
Disease location			0.057
L1	23 (53.5)	9 (29)	
L2	1 (2.3)	4 (12.9)	
L3	17 (39.5%)	14 (45.2%)	
L1-4	2 (4.7%)	3 (9.7%)	
L3-4	-	1 (3.2%)	
Behavior			
B1	1 (2.3)	4 (12.9)	0.071
B2	22 (51.2)	19 (61.3)	0.387
B3	20 (46.5)	8 (25.8)	0.07
Perianal disease	12 (27.9)	9 (29)	0.916
Smoking habits			0.635
Non-smoker	22 (52.4)	11 (44)	
Ex-smoker	8 (19)	9 (36)	
Current smoker	12 (28.6)	5 (20)	
Medication at index colonoscopy			
Thiopurines	23 (53.5)	25 (67.6)	0.200
Anti-TNFα	17 (39.5)	19 (51.4)	0.289
Obstructive symptoms	15 (35.7)	10 (27.8)	0.454
C-reactive protein	2.8 (0.3-18.4)	6.95 (0.2-35.5)	0.126
Calprotectin	103.5 (5.9-1356)	283 (166-321)	0.789
Lactoferrin	7.66 (1.05-204.4)	10.7 (7.1-22.7)	0.429
Need to repeat dilation	27 (62.8)	15 (40.5)	0.047

TNFα: Tumor necrosis factor α.

symptoms, serum/fecal biomarkers) were found to influence need for repeat dilation. After initial dilation, 4.6% of the patients (*n* = 2) required anastomotic stricture resection due to worsening or recurrence of symptoms. The median time to progression to surgery was 35 mo (33.4-36.7).

Non-anastomotic strictures

During the study period, a total of 37 CD patients (40.5% males, *n* = 15) underwent a total of 59 EBD sessions of non-anastomotic strictures (17 in the ileum, 13 in the ileocecal valve, 2 in the ascending colon, 1 in the transverse colon, 3 in the descending colon and 1 in the sigmoid colon) (Table 3). Twenty-seven percent of the patients (*n* = 10) had obstructive symptoms, with a median HBI of 1 (0-10). Technical success was achieved in all patients (*n* = 37). Repeat dilation was required in 40.5% (*n* = 15) of patients (long-term efficacy: 59.5%), with a median balloon dilation of 16.5mm (range 10-18). Technical success and improvement in obstructive symptoms were achieved in all patients without AEs. Following EBD, medical therapy was escalated in 18.9% of patients (*n* = 7; 4 began thiopurines and 6 began anti-TNF). Median time to second dilation was 368 d (range: 157-1705). Only 1 patient (3%) required surgical resection. No risk factors were found to influence need for repeat dilation.

Anastomotic strictures vs non-anastomotic strictures

Baseline characteristics of patients with anastomotic and non-anastomotic strictures are shown in Table 3. Follow-up since 1st dilation was longer in patients with anastomotic strictures. Patients with anastomotic strictures had a greater recurrence of stenosis (63% *vs* 41%, *P* = 0.047). In the univariate analysis, absence of thiopurine medication (OR = 3.1, 95%CI: 1.2-7.9, *P* = 0.019) and anastomotic strictures (OR = 2.5, 95%CI: 1.01-6.1, *P* = 0.049) were the only factors found to influence need for repeat dilation.

Asymptomatic and symptomatic cohorts

No statistical significant differences were found between asymptomatic and symptomatic cohorts when comparing baseline disease characteristics, medical therapy at time of dilation, disease activity,

Table 4 Baseline disease characteristics, endoscopic balloon dilation procedure, need for further dilations, escalation of medical therapy and need for surgery between asymptomatic and symptomatic cohorts *n* (%)

Characteristic	Asymptomatic strictures[1] (*n* = 53)	Symptomatic strictures[1] (*n* = 25)	*P* value
Female/male (*n*)	25/28	16/9	0.165
Disease duration in years (median; min-max)	17.9 (2.9-45.3)	19.9 (5.3-52.1)	0.955
Follow-up since 1st dilation in years (median; min-max)	3.1 (1.3-6.8)	3.9 (2.1-6.7)	0.068
Time between 1st and 2nd dilation in days (median; min-max)	668 (157-2074)	877 (152-2242)	0.790
Age at index colonoscopy (mean ± SD)	41.7 ± 13.8	43 ± 9.3	0.425
Montreal classification			
Age at diagnosis			0.998
A1	7 (14)	3 (13.6)	
A2	36 (72)	16 (72.7)	
A3	7 (14)	3 (13.6)	
Disease location			0.319
L1	22 (44)	9 (40.9)	
L2	5 (10)	-	
L3	21 (42)	9 (40.9)	
L1-4	1 (2)	4 (18.2)	
L3-4	1 (2)	-	
Behavior			0.833
B1	4 (8)	1 (4.5)	
B2	28 (56)	12 (54.5)	
B3	18 (36)	9 (40.9)	
Perianal disease	15 (30)	6 (27.3)	0.815
Smoking habits			0.761
Non-smoker	24 (52.2)	8 (42.1)	
Ex-smoker	8 (17.4)	4 (21.2)	
Current smoker	14 (30.4)	7 (36.8)	
Previous surgery (ileocecal resection/right hemicolectomy)	27 (50.9)	15 (60)	0.454
Medication at index colonoscopy			
Thiopurines	33 (62.3)	13 (52)	0.390
Anti-TNFα	21 (39.6)	14 (56)	0.175
Combo	13 (24.5)	9 (36)	0.293
C-reactive protein	6.2 (0.2-35.5)	3.2 (0.3-19.4)	0.351
Calprotectin	107 (18.2-837)	340 (5.9-1356)	0.449
Lactoferrin	7.1 (1.1-102.6)	37.6 (4.0-204)	0.071
Therapeutic success	52 (98.1)	25 (100%)	0.377
Balloon diameter (median; min - max)	18 (10-18)	18 (13.5-18)	0.201
Adverse events	0 (0)	1 (4)	0.129
Need to repeat dilation	27 (50.9)	15 (60)	0.454
Number of dilations (median; min - max)	2 (1-5)	2 (1-5)	0.463
Escalation of medical therapy after dilation	13 (27.7)	8 (34.8)	0.541
Need for surgery after dilation	2 (4)	1 (4.2)	0.973

[1]Two patients had no information regarding presence of obstructive symptoms (one in each group). TNFα: Tumor necrosis factor α.

EBD procedure, need for further dilations, escalation of medical therapy and need for surgery (Table 4).

DISCUSSION

CD is characterized by chronic, recurrent, transmural inflammation; intestinal strictures are believed to result from partial healing and localized fibrosis[18]. Strictures develop unpredictably after surgery[6]. EBD has emerged as a bridging tool for management of CD strictures with favorable success rates and efficacy[10].

In this study, we found that almost one-third of CD patients develop an anastomotic stricture after ileocecal resection/right hemicolectomy. The mean age at first dilation was 42.6 years, which reflects their etiology as a complication of the surgery. Longer intervals after surgery and higher lactoferrin levels were associated with anastomotic strictures; time until dilation was lower in patients with calprotectin levels > 83.35 μg/g and current/past history of smoking. Previous studies have not studied the development of anastomotic strictures after surgery, however, as a progressive disease, anastomotic strictures will be more likely over time. Both anastomotic and *de novo* strictures have either inflammatory and/or fibrotic elements. Healing occurs in a defined pattern during bouts of activity and remission, with progression to luminal narrowing leading to stricture formation[17]; on the other hand, elevated serologic markers probably reflect the inflammatory component of the neo terminal ileum instead of the presence of an anastomotic stricture. While there is controversy regarding the effect of smoking on the disease phenotype, literature supports smoking as a factor in complicated disease[19].

Regarding EBD, our technical success was 97.7%, similar to literature (88%-100%)[20] and similar to non-

anastomotic strictures (100%), however, this was not associated with a permanent stricture dilation, as 63% of patients with anastomotic strictures and 41% of those with non-anastomotic strictures required additional dilation over a 4.4-year period. The fibrotic pathology of anastomotic strictures may be responsible for the lower response rate of EBD for non-anastomotic strictures. Re-dilation was as technically successful, supporting the evidence that repeated dilations do not reduce the procedural efficacy[20,21]. The rate of major AEs, including bowel perforation and significant bleeding, has been reported to be between 2%-6%[1,8]. AEs have been attributed to balloon size (25 mm diameter), pressure used to dilate and number of dilations per session. Our data confirm safety of EBD for CD strictures. We had no serious AEs, which may be explained by the careful patient selection, fluoroscopy to evaluate stricture characteristics and monitoring, maximum balloon diameter of 18 mm, use of CO_2 insufflation, and endoscopists experience.

Long-term outcome following EBD varies. In our study, a long-term efficacy of 37.2% for anastomotic strictures and 59.5% for non-anastomotic strictures was achieved during a follow-up period of 4.4 years. Even though follow-up was longer, results were somewhat inferior to previous studies (52%-69%) when considering only anastomotic strictures. However, EBD in previous studies was symptom-driven, while in ours, EBD was performed for strictures that did not allow passage with a colonoscope, regardless of patients' symptoms. Besides, EBD delayed time until surgery, with only 3 patients (2 with anastomotic and 1 with non-anastomotic strictures) requiring surgery during follow-up period, suggesting a benefit of EBD. A recent pooled analysis reported a technical success, clinical success, long-term symptomatic and surgical recurrence rates of 89%, 81%, 48% and 29%, respectively[22]; these data are almost exclusively derived from retrospective cohort studies and may somewhat overestimate the actual benefit. In 2013, the European Crohn's and Colitis Organisation stated that EBD was safe and effective and allowed surgery to be avoided in CD patients with anastomotic strictures[23]. The overall technical success rate in the meta-analysis performed by Hassan *et al*[8] was 86% (71%-100%), while 41% of patients required repeated EBD allowing an overall long-term clinical efficacy (avoidance of surgery) rate of 58% during a median follow up of 33 mo.

Risk factors associated with need for subsequent dilation have been inconsistent. Longer disease duration was associated with a shorter time to repeat dilation[12]; technical success of dilation[8,24,25], length of stricture[8,26], and non-ulcerated stricture[27] were found to be associated with a successful procedure. In our study, the only factors found to influence need for repeat dilation (in the univariate analysis) were absence of thiopurine medications and anastomotic strictures. Escalation of medical therapy did not decrease the need for repeat dilation. Considering therapeutic strategy, Thienpont *et al*[10] reported no significant effect of systemic medical therapy after dilation on redilation or surgery, while Honzawa *et al*[28] found that prior use of immunomodulatory drugs improved the clinical outcome of EBD for intestinal strictures in patients with CD. Patients in our study may also have had more severe disease, as demonstrated by the majority receiving immunossupressants at the time of index colonoscopy (immunomodulators: 68.5%; biological therapy: 36.4%), as well as the high rate of repeat dilation. Despite the well-known deleterious association between tobacco use and CD activity, with increased risk of recurrence after surgery and EBD, we did not find any association between smoking and long-term outcome of EBD. Disease activity assessed by serologic markers, endoscopy, and clinical variables such as HBI, disease duration or time between surgery and dilation did not predict the need for repeat dilation, in accordance with previously published data[10,21].

It is controversial whether asymptomatic strictures should be endoscopically treated. Previous studies have found no correlation between patient´s symptoms and clinical scores and endoscopic/radiographic findings after intestinal resection[29-31]. In our study, only 2 patients presented with an HBI > 7 and only 27 patients complained of obstructive symptoms, despite 47 having an anastomotic stricture. Serum biomarkers did not correlate with endoscopic recurrence or presence of strictures, supporting the belief that using only symptoms or C-reactive protein levels to make treatment decisions may increase the risk of disease progression and AEs. Our group believes that dilation of strictures, despite symptoms, has impact on patients' management and disease course, allowing evaluation of disease activity and therapeutic adjustments. If EBD was not performed, a diagnosis of endoscopic recurrence would not have been possible in 33% of patients, all with normal biomarkers. On the other hand, we did not find any differences between asymptomatic and symptomatic cohorts regarding disease characteristics as well EBD peculiarities.

Limitations of our study include its retrospective nature, being conducted in a tertiary referral center (with referral or selection bias), lack of a control group (medical and surgical therapy), uncertainty of the degree of luminal narrowing caused by inflammation *vs* fibrosis and escalation of medical treatment biased toward those having active IBD.

In conclusion, smoking habits and longer disease duration after surgery are associated with a higher risk of anastomotic strictures. EBD is a feasible, simple, effective and safe alternative to surgery, with the possibility of being repeated as needed, with excellent symptomatic response, as well as good short-term and long-term outcomes, postponing or avoiding surgery. Considering that a significant number of patients with significant strictures remain asymptomatic with normal

biomarkers, and the fact that the disease continues to evolve proximal to the strictures, we believe EBD should be considered for all strictures not transposable by a colonoscope, regardless of the presence or absence of symptoms, in order to adjust treatment in an attempt to alter the natural history of the disease. Thus, EBD is useful not only for symptom resolution but also for evaluating mucosal healing.

ARTICLE HIGHLIGHTS

Research background

Strictures in Crohn's disease (CD) develop during the course of the disease or as the presenting feature. More than half of CD patients will need surgery within the first 10 years of diagnosis. Medical therapy for stricture management is limited due to the fibrotic nature. Endoscopic balloon dilation (EBD) has been proposed as a safe and effective therapeutic intervention for CD strictures, particularly for ileocecal and anastomotic strictures.

Research motivation

Data on long term efficacy and safety of EBD are limited due to lack of long-term outcome and small cohorts. Up to now there are also some uncertainties regarding the factors associated with long term success rate. Smoking status and disease activity status at the time of dilation may affect outcome of stricture dilation, though many studies are limited by short follow-up durations and small cohorts. Furthermore, as the primary therapeutic goal of CD has shifted from clinical remission to achieving mucosal healing, it may be important to access the mucosa proximal to strictures to evaluate disease recurrence and escalate therapy if needed.

Research objectives

This study aimed to evaluate anastomotic stricture development after intestinal resection in CD and demonstrate long-term efficacy and safety center of EBD in CD anastomotic and *de novo* strictures in a large referral centre cohort and determine the impact of dilation on the diagnosis of subclinical postoperative endoscopic recurrence.

Research methods

CD patients who had undergone ileocecal resection/right hemicolectomy referred for endoscopic evaluation between March 2010 to February 2015 were included in this study. CD patients with non-anastomotic strictures who underwent EBD during the study period were included as a control group. EBD was performed for strictures that would not allow passage with a colonoscope, regardless of patients' symptoms. Technical success was defined as the ability to pass the colonoscope through the stricture into the neoileum following dilation. Clinical success was defined as improvement of obstructive symptoms (in symptomatic patients). All patients who underwent dilation were endoscopically reevaluated 6-12 mo later. Long-term efficacy was defined as avoidance of surgical resection or repeat dilation after the initial dilation. Patients were followed until stricture resection, last clinic follow-up, or censor date of March, 2017. Escalation of medical therapy was defined as initiation of a thiopurine or anti-TNF within 6 months of first dilation, as determined by global physician assessment.

All data were prospectively collected in a database created for this purpose. After a 5 year follow up period all data were arranged, processed and analyzed with SPSS® v.24.0 data (Statistical Package for Social Sciences).

Research results

In this study we found that almost one-third of CD patients developed an anastomotic stricture after ileocecal resection/right hemicolectomy. Longer periods between surgery and *index* colonoscopy and higher lactoferrin levels were associated with the presence of stricture after surgery. Calprotectin levels > 83.35 μg/g and current or past history of smoking were associated with a shorter time until need for dilation (HR = 3.877, 95%CI: 1.480-10.152 and HR = 3.041, 95%CI: 1.213-7.627). Technical success of EBD was 97.7% and 100% for anastomotic and non-anastomotic strictures, respectively, and 63% and 41% of the patients needed repeat dilation during the 4.4-year follow-up. Anastomotic strictures had a greater need for repeat dilation (63% *vs* 41%, P = 0.047). No differences were found between asymptomatic and symptomatic cohorts. Disease recurrence was diagnosed only after EBD in a third of patients.

Research conclusions

EBD is a feasible, simple, effective and safe alternative to surgery, with the possibility of being repeated as needed, with excellent symptomatic response, as well as good short-term and long-term outcomes, postponing or avoiding surgery. Considering that a significant number of patients with significant strictures remain asymptomatic with normal biomarkers, and the fact that the disease continues to evolve proximal to the strictures, we advocate EBD for all strictures regardless of the presence or absence of symptoms, in order to adjust treatment in an attempt to alter the natural history of the disease. Thus, EBD is useful not only for symptom resolution but also for evaluating mucosal healing.

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MONITORING CROHN´S DISEASE ACTIVITY: ENDOSCOPY, FECAL MARKERS AND CT ENTEROGRAPHY

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Abstract:	Background: The treatment goal of Crohn´s disease (CD) has moved towards achieving mucosal healing, resolution of transmural inflammation and normalization of biomarkers. The purpose of this study was to evaluate how well CT enterography (CTE) and fecal calprotectin (FC) correlated with endoscopic activity, in newly diagnosed CD patients and after one year of therapy. Methods: Consecutive patients with newly diagnosed CD diagnosis were evaluated by endoscopy, CTE and FC at diagnosis and 12 months after beginning immunosuppression. Endoscopic severity was assessed using the simplified endoscopic score for Crohn´s disease (SES-CD). Biomarkers, clinical indexes, and fecal calprotectin were recorded on the day of ileocolonoscopy at diagnosis and one-year after diagnosis. We adapted a CTE score for disease activity based on radiological signs of inflammation (mural thickness, mural hyperenhancement, mesenteric fat proliferation, mesenteric fat densification, comb sign, presence of strictures, fistulas, abscesses, ascites and lymphadenopathy). Correlations between endoscopy, CTE, and FC were assessed using Spearman's rank correlation. Results: Twenty-nine patients (48% female; men age 30 ± 9.5 years) were included in this prospective cohort. CTE findings significantly correlated with endoscopic findings. Endoscopic remission at one-year follow-up significantly correlated with improvement in mural hyperenhancement (p=0.004), mesenteric fat densification (p=0.001), comb sign (p=0.004), and strictures (p=0.008) in CTE. None of the CTE findings improved in patients without endoscopic remission. FC correlated with SES-CD (r=0.696, p<0.001) and with CTE features of inflammation (r=0.596, p<0.001). A cut-off of 100 ug/g predicted endoscopic remission

	with 92% sensitivity, 65% specificity and 83% accuracy (AUC 0.878, p<0.001). Conclusion: CTE findings and FC levels correlated with endoscopic activity in CD both at diagnosis and at one-year follow-up. These two noninvasive markers of disease activity may be used as an alternative to endoscopy to monitor disease response to therapy.

MONITORING CROHN´S DISEASE ACTIVITY: ENDOSCOPY, FECAL MARKERS AND CT ENTEROGRAPHY

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SL contributed to the study design and conception, data collection and drafting of the manuscript. PA contributed to data collection and statistical analysis. JA performed all laboratorial procedures. ERP contributed to statistical analysis. RC and IR performed all radiological analysis GM supervised the study and revised the manuscript, FM contributed to study design and conception, supervised the study and revised the manuscript,.

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Abbreviations:

CD: Crohn's disease; CTE: computed tomography enterography; ER: endoscopic remission; FC: fecal calprotectin; IBD: inflammatory bowel disease; HBI: Harvey-Bradshaw index; HR: histological remission; MRE: resonance enterography; PPV: positive predictive value; NPV: negative predictive value, SES-CD: simplified endoscopic score for Crohn´s disease; TNF: tumour necrosis factor;

ABSTRACT

Background: The treatment goal of Crohn´s disease (CD) has moved towards achieving mucosal healing, resolution of transmural inflammation and normalization of biomarkers. The purpose of this study was to evaluate how well CT enterography (CTE) and fecal calprotectin (FC) correlated with endoscopic activity, in newly diagnosed CD patients and after one year of therapy.

Methods: Consecutive patients with newly diagnosed CD diagnosis were evaluated by endoscopy, CTE and FC at diagnosis and 12 months after beginning immunosuppression. Endoscopic severity was assessed using the simplified endoscopic score for Crohn´s disease (SES-CD). Biomarkers, clinical indexes, and fecal calprotectin were recorded on the day of ileocolonoscopy at diagnosis and one-year after diagnosis. We adapted a CTE score for disease activity based on radiological signs of inflammation (mural thickness, mural hyperenhancement, mesenteric fat proliferation, mesenteric fat densification, comb sign, presence of strictures, fistulas, abscesses, ascites and lymphadenopathy). Correlations between endoscopy, CTE, and FC were assessed using Spearman's rank correlation.

Results: Twenty-nine patients (48% female; men age 30 ± 9.5 years) were included in this prospective cohort. CTE findings significantly correlated with endoscopic findings. Endoscopic remission at one-year follow-up significantly correlated with improvement in mural hyperenhancement (p=0.004), mesenteric fat densification (p=0.001), comb sign (p=0.004), and strictures (p=0.008) in CTE. None of the CTE findings improved in patients without endoscopic remission. FC correlated with SES-CD (r=0.696, p<0.001) and with CTE features of inflammation (r=0.596, p<0.001). A cut-off of 100 ug/g predicted endoscopic remission with 92% sensitivity, 65% specificity and 83% accuracy (AUC 0.878, p<0.001).

Conclusion: CTE findings and FC levels correlated with endoscopic activity in CD both at diagnosis and at one-year follow-up. These two noninvasive markers of disease activity may

be used as an alternative to endoscopy to monitor disease response to therapy.

KEYWORDS: Crohn's disease; ileocolonoscopy; fecal calprotectin; CT Enterography; mucosal healing; disease monitoring

INTRODUCTION

Crohn's disease (CD) is a chronic relapsing inflammatory bowel disease (IBD) characterised by transmural inflammation often affecting multiple sites of the gastrointestinal tract (1). In recent decades, treatment goals in CD have evolved greatly (2); although symptomatic control was once considered the goal of therapy, with the introduction of anti-tumour necrosis factor (TNF)-α agents in the 1990's, endoscopic remission (ER) and histological remission (HR) both reflecting mucosal healing have become accepted therapeutic targets (3, 4). A wealth of data suggests that mucosal healing may alter the natural course of disease by decreasing the rates of hospitalisation and reducing the need for surgery (5, 6). However, since CD often affects the small bowel beyond the terminal ileum, ileocolonoscopy alone may be inadequate for the correct evaluation of mucosal inflammation in CD (7).

Cross-sectional imaging including computed tomography enterography (CTE) and magnetic resonance enterography (MRE) have been ~~recently~~ introduced into clinical practice and have emerged as preferred modalities for the evaluation of small bowel involvement in CD (8). These imaging modalities not only allow the accurate assessment of small bowel disease activity, extent, and location, but also aid in diagnosing extraluminal manifestations and CD complications during a single examination (8, 9). CTE and MRE perform similarly in the assessment of disease (10), but interobserver agreement and image quality may be superior with CTE (11, 12). In a preliminary study, Hara et al have reported that CTE may also have

the potential for longitudinal disease monitoring, noting its reliability to predict CD progression or regression (13). In addition, a more recent retrospective study showed that 63% of patients had a significant radiological response to anti-TNF-α agents as assessed by serial CTEs (14). However, this study was marred by its retrospective nature and by the fact that repeated CTE was performed in symptomatic patients only (14), and therefore the real accuracy of CTE to assess therapeutic responses remains to be prospectively assessed.

Fecal calprotectin (FC), a 36-kDa calcium- and zinc-binding protein complex derived from leukocytes infiltrating the intestinal wall, has recently emerged as a noninvasive biomarker of intestinal inflammation (15). Several studies have shown that FC reflect endoscopic disease activity in CD, predicting endoscopic inflammation and being a surrogate marker of mucosal healing (16, 17). However, the most appropriate cut-off value for FC to predict endoscopic activity in patients with clinical remission has yet to be determined.

Despite their widespread availability in clinical practice, the performance of CTE and FC compared to endoscopy as diagnostic tools and/or as measures to evaluate therapeutic response in CD are yet to be determined. This prospective study was designed to evaluate the correlation between endoscopic disease activity, fecal markers and CTE findings of inflammatory activity in newly diagnosed CD patients and one year after initiation of immunosuppressive therapy.

MATERIAL AND METHODS

Patients

Consecutive newly diagnosed adult CD patients were prospectively enrolled between January 2013 and October 2014 at Centro Hospitalar Sao Joao (Porto, Portugal). Patients were included if the following criteria were met: 1. presence of a definitive diagnosis of CD based on accepted clinical, radiological, endoscopic and histological criteria criteria (18) ; 2.

requiring therapy with steroids, azathioprine, and/or anti-TNF-α agents; and 3. presence of endoscopic activity, defined by a Simplified Endoscopic Activity Score for CD (SES-CD) ≥ 3 (19). Patients younger than 18 years-old, who were pregnant, needed immediate surgery, or did not have endoscopically active disease at the time of enrollment were excluded.

Eligible patients underwent ileocolonoscopy, CTE, and FC determination at diagnosis and at 1 year of follow-up. Time between ileocolonoscopy and CTE was less than 4 weeks and no therapeutic changes were performed during that time period. Considering strict inclusion and exclusion criteria, sample size at the end of recruitment period was 29 patients.

Disease phenotype was determined according to Montreal classification, based on age at onset, location and behaviour, with perianal and upper gastrointestinal tract involvement as additional modifiers (20). Gender, age, age at onset, disease location, disease behaviour, perianal disease, smoking habits, Harvey-Bradshaw index (HBI) and laboratory workup at diagnosis and at 1 year of follow-up were recorded. A HBI of less than 5 was considered as clinically inactive disease, and a 2 points drop on HBI was considered as disease improvement.

Endoscopy

Ileocolonoscopy was performed under propofol sedation by a single board-certified gastroenterologist (SL) experienced in the endoscopic examination of CD patients. All patients had the distance of the ileum scoped specified in their endoscopic report, with a median distance of 10 cm. A solution of polyethyleneglycol was used on the night before, for bowel preparation. Endoscopic lesions were assessed using the SES-CD (19). Endoscopic remission was defined in the protocol as a SES-CD ≤ 3.

CTE technique

All CTE were performed at our institution, using a 64 row-multidetector CT Siemens Somaton Sensation (Erlangen, Germany) scanner under American College of Radiology guidelines for CT enterography. Axial, coronal and sagittal images 2 mm in thickness were obtained.

Patients were asked to be nil by mouth for 6 hours before the procedure. Just before the scanning patients were asked to drink 2000 mL of water with 40g of mannitol (200 ml of Baxter solution with 20% of mannitol diluted with 1800 cc of water), over 60 minutes, at a steady rate of approximately 500ml every 15 minutes. Contrast enhanced CT images were acquired at enteric phase 50 seconds after intravenous injection of 80 mL of ioversol (Optiray 320; Mallinckrodt Canada, Quebec, Canada). CTE images were analysed using transverse and multiplanar views. Multiplanar images were reconstructed with 2 mm of slice thickness.

CTE image interpretation

Images were evaluated using the PACS system (SECTRA AB, Sweden). Radiological interpretation and scoring was performed by a senior radiologist with more than 8 years of experience dedicated to IBD imaging. He was blinded to all the clinical, laboratory and endoscopic data and was asked to identify all bowel segments with signs of inflammation. Six CT signs of active CD (mural thickness, mural hyperenhancement, mesenteric fat proliferation, mesenteric fat densification, comb sign, presence of strictures) were evaluated in 5 predefined ileocolonic segments (ileum, right colon, transverse colon, left colon and rectum) (21).

Mural thickening was assumed for a small bowel wall thickness of greater than 3 mm in a distended loop. Stenosis were suggested when there is visual luminal narrowing and upstream bowel dilation >30 mm. Each variable was scored as either 0 (absent) or 1 (present) per segment. In addition, fistulas, abscesses, ascites and lymphadenopathy were globally scored as either 0 (absent) or 1 (present). Thus, the total CTE score was 34.

Fecal calprotectin

Stool samples were collected the day before beginning bowel preparation (preferably from the first stool in the morning) and then kept in the fridge until being brought to the hospital. Within a maximum of 7 days after collection, stools were extracted in accordance with the manufacturer's instructions, using a "Faecal sample preparation kit" (Roche Diagnostics, Mannheim, Germany). Sample extracts were stored at -80ºC until the assays were performed at the Department of Pharmacology and Therapeutics, Faculty of Medicine of the University of Porto. Samples were thawed and analyzed using a commercially available fluoroenzyme immunoassay (EliA Calprotectin®; Thermo Fisher Scientific, Freiburg, Germany).

Statistical analysis

Categorical variables were described through absolute and relative frequencies and continuous variables were described as mean and standard deviation, median, percentiles, minimum and maximum. McNemar tests were used to compare paired samples. The Spearman's rank correlation coefficient was applied for assessing the correlations between CTE score and SES-CD, HBI, FC as well as other laboratory parameters.

Receiver operating characteristic (ROC) analysis was applied for determining the optimal cut-off values with the sensitivity and specificity based on the endoscopic remission (SES-CD≤3). All the reported p values were two-sided, and p values of <0.05 were considered statistically significant. All data were arranged, processed and analysed with Statistical Package for Social Sciences (SPSS ®) v.20.0 data (SPSS Inc., Chicago, IL, USA).

Ethical considerations

This study was conducted according to the Declaration of Helsinki. The study protocols were approved by the Ethics Committee of Centro Hospitalar São João, Porto, Portugal on 27th February 2012 (ethics approval number 145/12 and 84/12). All patients gave informed consent to participate in this study in accordance with the local institutional board regulations.

RESULTS

Population

A total of 29 newly diagnosed CD patients were enrolled. Table 1 depicts baseline demographic characteristics. Fourteen (48%) patients were female with a mean age of age 30 ± 9.5 years. At diagnosis, nearly all patients (n=28, 97%) were aged between 17 and 40 years-old. Nineteen (65.6%) patients had exclusively ileal involvement (L1) and 14 (48%) had non-stricturing non-penetrating behaviour (B1). Seven (24%) had evidence of perianal disease. Ten (34%) patients were smokers while 2 (7%) were former smokers. All patients had clinical and endoscopically active disease at baseline, with a median SES-CD score of 10 (7–16).

All patients received systemic steroids (equivalent to prednisolone 1mg/Kg) at diagnosis after all the contraindications were solved (e.g. abscesses in 3 patients). 97% (n=28) of patients were started on azathioprine, while 59% (n=17) started biologic therapy (infliximab: 11; adalimumab: 6), having started them at median of 9 and 162 days after diagnosis, respectively.

At one-year follow-up 24 (83%) patients were in clinical remission, with a median HBI of 1 (0.0-2.0) and 19 (66%) patients were in endoscopic remission. Compared to baseline, median values of haemoglobin and albumin were significantly higher at 1-year of follow-up, while C-reactive protein levels were significantly lower (Table 2).

Comparison of colonoscopy and CTE findings

At baseline, all patients had ileal involvement at CTE, with 59% (n=17) having exclusively ileal involvement, while 24% (n=7) had two different locations involved, 10% (n=3) three different locations involved and 7% (n=2) 4 different locations involved. Seventeen percent of the patients (n=5) had disease proximal to the reach of the colonoscope.

CTE findings at baseline showed bowel thickening and hyperenhancement in 29 (100%) and 28 (97%) patients, respectively. Twenty-one (72%) patients presented fat densification and comb sign of at least one bowel segment. Strictures were observed in 18 (62%) patients. Fistulas were identified in 9 (31%) patients and abscesses in 3 (10%).

Most of the CTE findings improved at one year of follow-up. Baseline and at one year of follow-up individual CTE findings are shown in Table 3. An example of a patient' endoscopic findings and corresponding CTE images at baseline and at one year of follow-up is shown in figure 2 and 3, respectively.

Endoscopic remission at one-year follow up was significantly associated with improvement in mural hyperenhancement (p=0.004), mesenteric fat densification (p=0.001), comb's sign (p=0.004), and strictures (p=0.008) in CTE. None of the CTE findings improved in patients without endoscopic remission (Table 3). Five patients in endoscopic remission showed complete disappearance of disease signs at CTE.

CTE score and correlation with clinical parameters

At diagnosis, the median CTE score was 7.0 (4.5-10.0), while at 1-year follow-up it decreased to 3.0 (0.0-6.5; p<0.001). When considering examinations both at baseline and 1-year follow-up together (totalling 58 of each procedures), the median value of CTE was 5.5 (2.8 – 8.0). The CTE score level showed significant correlation with either HBI (r_s=0.787,

p<0.001), SES-CD score (r_s=0.746, p<0.001), C-reactive protein (r_s=0.671, p<0.001), and haemoglobin (r_s=-0.580, p<0.001).

Fecal calprotectin and correlation with clinical parameters and CTE findings/CTE score

At diagnosis, the median FC value was 986.5 (361.8-3175.8), while at 1-year follow-up it decreased to 53.0 (23.8-648.5; *p*<0.001). When considering FC determinations both at baseline and 1-year follow-up together (totalling 58 determinations), the median value of FC was 499.0 (52.7 – 1537.5). FC significantly correlated with HBI (r_s=0.450, p=0.001), SES-CD score (r_s=0.696, p<0.001), C-reactive protein (r_s=0.609, p<0.001), CTE score (r_s=0.596, p<0.001) and haemoglobin (r_s=-0.383, p=0.003). The location of the disease did not influence the accuracy of FC (L1 [r_s=0.695, p<0.001] and L3 [r_s=0.678, p<0.001]). We found that FC significantly correlated with the same CTE variables that reflected endoscopic activity: bowel hyperenhancement (r_s=0.458, p<0.001), fat densification (r_s=0.508, p<0.001), comb´s sign (r_s=0.437, p=0.001), and strictures (r_s=0.329, p=0.012); in addition, FC also correlated with the presence of lymphadenopathy (r_s=0.426, p=0.001). CTE findings distributed per ileocolonic segments at baseline and after 1 year of follow-up are depicted in a supplementary table.

Higher values of FC significantly correlated with a higher number of CTE findings per ileocolonic segment (r_s=0.521, p<0.001).

Fecal calprotectin, CTE and endoscopic remission

Nineteen patients were in endoscopic remission at one-year follow-up. Patients in endoscopic remission had lower C-reactive protein levels (3mg/L [0.2-16.4] *vs* 20.1mg/L [0.7-125.7], p<0.001), lower calprotectin levels (42.1 [2.6-1208] *vs* 857 [26.7-10600], p<0.001), a HBI ≤4 (94.7% *vs* 15.4%, p<0.001) and a CTE score ≤3 (73.7% *vs* 12.8%, p<0.001).

In ROC analyses (Figures 1&2), endoscopic remission at ileocolonoscopy was predicted by a CTE score ≤3 with 87.2% sensitivity, 73.7% specificity, 87.2% positive predictive value (PPV), 73.7% negative predictive value (NPV) and 82.8% accuracy (AUCROC 0.866,

p<0.001 95%CI 0.761-0.970) and by a calprotectin value<100 ug/g with 92.1% sensitivity, 65.0% specificity, 83.3% PPV, 81.3% NPV and 82.7% accuracy (AUCROC 0.878, p<0.001 95%CI 0.781-0.976).

With the aim of simplifying the application of these results on a daily basis so a likelihood of endoscopic remission at ileocolonoscopy could be achieved, a model combining FC value and CTE score was created by applying the following logistic function:

$$\frac{\exp(-14.685 + 1.227 * \text{Haemogl. level} - 0.004 * \text{Calprot. level} - 2.422 * \text{CTE score})}{1 + \exp(-14.685 + 1.227 * \text{Haemogl. level} - 0.004 * \text{Calprot. level} - 2.422 * \text{CTE score})}$$

Assuming a sensitivity of 89.5% and a specificity of 89.7%, we consider the cut-off of -0.3146 as clinically relevant, with a PPV of 94.6%, a NPV of 80.9% and an accuracy of 89.6%, regarding likelihood of endoscopic remission at ileocolonoscopy (AUROC 0.946; 95% CI [0.892–1.000]).

DISCUSSION

In this study we show that CTE findings and FC levels mirror endoscopic activity in newly diagnosed CD patients. In addition, both CTE findings and FC levels are able to predict endoscopic remission one year after therapy, suggesting that these two noninvasive markers of disease activity may be used as an alternative to endoscopy to monitor disease response to therapy.

As the paradigm in CD treatment has shifted from clinical response to "bowel healing" (mucosal and transmural healing), it is now essential to define the best way to monitor disease activity. Attempts to correlate outcomes with radiological signs of inflammation have produced variable findings (22-26). However, most studies focused on mural findings rather than on mesenteric signs of inflammation(12). It is known that at least some of the mural thickening observed despite endoscopic remission may not be due to active inflammation, but rather histological alterations secondary to transmural healing (27, 28). We have therefore adapted a CTE scoring system that factored also features such as mural hyperenhancement, mesenteric fat proliferation and densification, and the comb sign, since these CTE findings are closely related to inflammation (9). In this work we were able to establish a strong correlation not only between mural findings but also mesenteric findings of inflammation (comb sign and fat densification) and endoscopic activity defined by SES-CD. Previous studies (25, 27) have also described the comb sign, enlarged lymph nodes and increased fat density as good markers of endoscopic and histological activity. These studies suggested that the CTE variables associated with more severe endoscopic disease were mesenteric in origin rather than mural. In line with these findings, here we shown that fat densification, comb sign, and mural hyperenhancement are the best predictors of disease activity in CD. In addition, we found a significant reversal in CTE signs of inflammation one year after treatment, reflecting disease response to immunosuppressive therapy. This radiological

improvement correlated significantly with clinical response as assessed by the HBI. Lastly, CTE score accurately predicted endoscopic remission.

On the other hand, FC has proved to correlate with disease activity and to be a good predictor of disease relapse and recurrence (29-31). A better correlation between SES-CD and FC than with serological markers has also been demonstrated (17, 32). Our group has recently shown that FC performed better than C-reactive protein in predicting endoscopic activity in the post-operative setting (33). In the current study, we show that FC significantly correlated with both endoscopic activity and CTE findings. Defining endoscopic remission as a SES-CD ≤3 , we found FC to have 92% sensitivity and 65% specificity for predicting endoscopic remission at a cut-off value of 100 ug/g. Interestingly, disease location seemed not influence the diagnostic performance of FC.

One limitation that could be pointed out to this study is the use of CTE to monitor CD, regarding radiation concern. Although MRE has emerged as a non-ionizing alternative method to CTE (34) not all centers have MRE readily available. CTE is cheaper, more readily accessible, faster, with higher spatial resolution and better tolerated by patients. CTE may even be superior compared to MRE in terms of image quality and interobserver agreement. Nowadays there are several strategies available to reduce radiation dose exposure, with no compromise of diagnostic accuracy. Nevertheless, cumulative radiation exposure of CD patients undergoing repeated CT examination needs to be carefully considered.

Another limitation of this study is the single reader analysis of CTE images, as we know that CTE interpretation is subjected to interobserver variation, the use of a CTE score that was not yet validated (but actually no CTE score is validated) and also the relative small simple size.

In conclusion, FC and CTE are good markers of disease activity in CD. In this group of newly diagnosed patients we found a good correlation between FC and SES-CD and CTE score, and between SES-CD and CTE score. Both FC and CTE score significantly improved

at one year of follow-up after beginning immunosuppressive therapy and strongly correlated with endoscopic findings. A CTE score ≤ 3 points and a FC < 100 ug/g accurately predicted endoscopic remission at one year follow up. Therefore, CTE and FC could be used as alternatives to endoscopic evaluation in newly diagnosed CD patients to monitor response to therapy.

DISCLOSURES

- Susana Lopes have no conflicts of interest or financial ties to disclose.
- Patrícia Andrade have no conflicts of interest or financial ties to disclose.
- Joana Afonso have no conflicts of interest or financial ties to disclose.
- Rui Cunha have no conflicts of interest or financial ties to disclose.
- Eduardo Rodrigues-Pinto have no conflicts of interest or financial ties to disclose.
- Isabel Ramos have no conflicts of interest or financial ties to disclose.
- Guilherme Macedo have no conflicts of interest or financial ties to disclose.
- Fernando Magro have no conflicts of interest or financial ties to disclose.

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FIGURE LEGENDS:

Figure 1: Study flowchart.

ADA: adalimumab; CD: Crohn's disease; IFX: infliximab; TNF: tumor necrosis factor;

Figure 2: Endoscopic findings at baseline (A and B) and corresponding CTE images (C, D, E and F).

Figure 3: Endoscopic findings after one year of follow up (A and B) and corresponding CTE images (C and D).

Figure 4: ROC curves for CTE score (A) and FC (B) for discriminating between endoscopic recurrence and remission.

Table 1. Baseline characteristic of Crohn's disease (CD) patients

Characteristics	**CD (n=29)**
Women, n (%)	14 (48.3)
Age at diagnosis, median, years (IQR)	30.0 (24.5-35.5)
Montreal classification	
Age, n (%)	
A2 (17-40 years)	28 (96.6)
A3 (>40 years)	1 (3.4)
Location, n (%)	
L1 (ileal)	19 (65.5)
L3 (ileocolonic)	10 (34.5)
L4 (upper gastrointestinal tract)	5 (17.2)
Behaviour, n (%)	
B1 (non stricturing, non penetrating)	14 (48.3)
B2 (stricturing)	6 (20.7)
B3 (penetrating)	9 (31.0)
Perianal disease, n (%)	7 (24.1)
Smoking, n (%)	
Never	17 (58.6)
Current	10 (34.5)
Former	2 (6.9)
Faecal calprotectin, median, µg/g (IQR)	986.5 (361.8-3175.8)
SES-CD, median (IQR)	10.0 (7.0-16.0)
CTE, median (IQR)	7.0 (4.5-10.0)
Number of segments involved in CTE, n (%)	
1	17 (58.6)
2	7 (24.1)
3	3 (10.3)
4	2 (6.9)
Harvey-Bradshaw index, n (%)	
Mild (5-7)	17 (58.6)
Moderate (8-15)	11 (37.9)
Severe (>15)	1 (3.4)
Follow up, median, months, (IQR)	33.0 (26.0-41.0)

CTE, Computed tomography enterography; IQR, interquartile range; SES-CD, Simple endoscopic score for Crohn's disease;

Table 2. Laboratory workup, SES-CD and CTE score changes between baseline and at 1 year of follow-up

	Baseline (95% CI)	At 1 year of FU (95% CI)	p-value
Hemoglobin	12.4 (11.8 – 12.9)	14.0 (13.6 – 14.5)	<0.001
Albumin	33.8 (31.1 – 36.5)	42.1 (40.1 – 44.0)	<0.001
C-reactive protein	43.0 (28.7 – 57.4)	7.4 (1.9 – 12.8)	0.01
Calprotectin	2335.2 (1170.6 – 3499.7)	542.4 (175.7 – 909.1)	0.005
SES-CD score	13.4 (10.1 – 16.7)	3.9 (1.9 – 6.0)	<0.001
CTE score	8.1 (6.3 – 9.9)	3.9 (3.4 – 5.4)	<0.001

FU: Follow-up; SES-CD: Simple Endoscopic Score for Crohn's Disease; CTE: computed tomography enterography

Table 3. Changes in CTE findings between baseline and 1 year of follow-up considering endoscopic activity

	All patients (n=29)			Endoscopic remission (n=19)			Endoscopic activity (n=10)		
	Baseline	**1 year of FU**	**p-value**	**Baseline**	**1 year of FU**	**p-value**	**Baseline**	**1 year of FU**	**p-value**
Mural thickness	100% (n=29)	79.3% (n=23)	-	100% (n=19)	68.4% (n=13)	-	100% (n=10)	100% (n=10)	1
Mural hyperenhancement	**96.6% (n=28)**	**62.1% (n=18)**	**0.002**	**94.7% (n=18)**	**47.4% (n=9)**	**0.004**	100% (n=10)	100% (n=10)	1
Mesenteric fat proliferation	65.5% (n=19)	58.6% (n=17)	0.687	57.9% (n=11)	47.4% (n=9)	0.687	80% (n=8)	80% (n=8)	1
Mesenteric fat densification	**72.4% (n=21)**	**20.7% (n=6)**	**<0.001**	**68.4% (n=13)**	**10.5% (n=2)**	**0.001**	80% (n=8)	40% (n=4)	0.219
Comb's sign	**72.4% (n=21)**	**34.5% (n=10)**	**0.001**	**63.2% (n=12)**	**15.8% (n=3)**	**0.004**	90% (n=9)	70% (n=7)	0.5
Strictures	**62.1% (n=18)**	**31% (n=9)**	**0.004**	**57.9% (n=11)**	**15.8% (n=3)**	**0.008**	70% (n=7)	60% (n=6)	1
Lymphadenopathy	27.6% (n=8)	13.8% (n=4)	0.125	10.5% (n=2)	0% (n=0)	-	60% (n=6)	40% (n=4)	0.5
Fistulas	**31% (n=9)**	**10.3% (n=3)**	**0.031**	31.6% (n=6)	10.5% (n=1)	0.125	30% (n=3)	10% (n=1)	0.5
Ascites	10.3% (n=3)	0% (n=0)	-	5.3% (n=1)	0% (n=0)	-	20% (n=2)	0% (n=0)	-
Abscesses	10.3% (n=3)	0% (n=0)	-	5.3% (n=1)	0% (n=0)	-	20% (n=2)	0% (n=0)	-

Supplementary table: CTE findings distributed per ileocolonic segments at baseline and after 1 year of follow-up

	Ileum n(%)		Right colon n(%)		Transverse colon n(%)		Left colon n(%)		Rectum n(%)	
	Baseline	**1 year of FU**	**Baseline**	**1 year of FU**	**Baseline**	**1 year of FU**	**Baseline**	**1 year of FU**	**Baseline**	**1 year of FU**
Mural thickness	29 (100.0)	23 (79.3)	3 (10.3)	2 (6.9)	4 (13.8)	2 (6.9)	5 (17.2)	1 (3.4)	0 (0.0)	0 (0.0)
Mural hyperenhancement	28 (96.6)	18 (62.1)	3 (10.3)	2 (6.9)	4 (13.8)	2 (6.9)	5 (17.2)	1 (3.4)	0 (0.0)	0 (0.0)
Mesenteric fat proliferation	19 (65.5)	17 (58.6)	0 (0.0)	1 (3.4)	1 (3.4)	1 (3.4)	3 (10.3)	1 (3.4)	0 (0.0)	0 (0.0)
Mesenteric fat densification	21 (72.4)	6 (20.7)	3 (10.3)	0 (0.0)	3 (10.3)	1 (3.4)	3 (10.3)	1 (3.4)	0 (0.0)	0 (0.0)
Comb's sign	21 (72.4)	10 (34.5)	2 (6.9)	0 (0.0)	4 (13.8)	0 (0.0)	5 (17.2)	0 (0.0)	0 (0.0)	0 (0.0)
Strictures	18 (62.1)	9 (31.0)	1 (3.4)	0 (0.0)	2 (6.9)	0 (0.0)	3 (10.3)	0 (0.0)	0 (0.0)	0 (0.0)

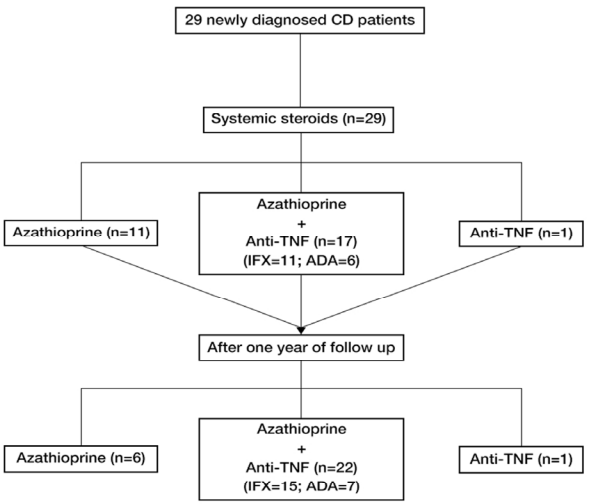

Figure 1: Study flowchart.
ADA: adalimumab; CD: Crohn's disease; IFX: infliximab; TNF: tumor necrosis factor;

143x138mm (150 x 150 DPI)

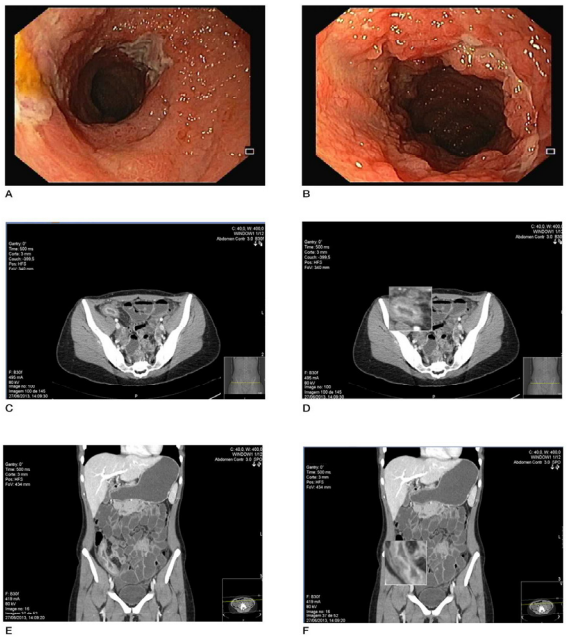

Figure 2: Endoscopic findings at baseline (A and B) and corresponding CTE images (C, D, E and F).

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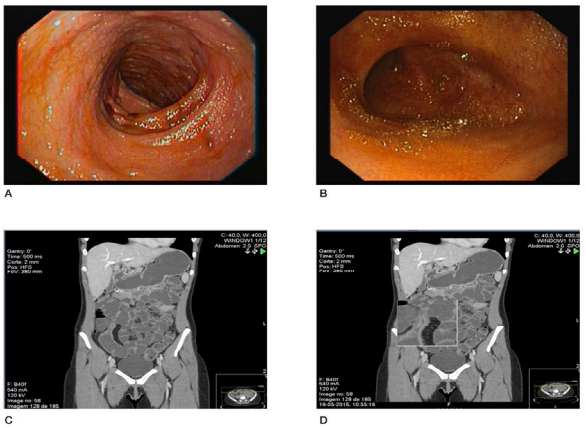

Figure 3: Endoscopic findings after one year of follow up (A and B) and corresponding CTE images (C and D).

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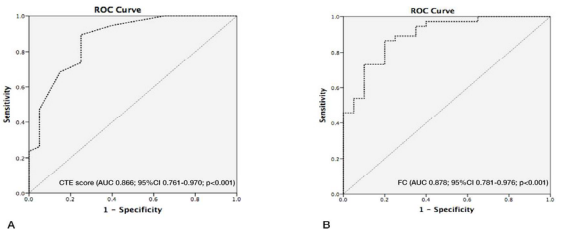

Figure 4: ROC curves for CTE score (A) and FC (B) for discriminating between endoscopic recurrence and remission.

304x123mm (150 x 150 DPI)

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Transmural healing in Crohn's disease: Beyond mural findings

Dear Editor,

We read with great interest the article by Castiglione et al. [1] reporting the rate of transmural healing (TH) in Crohn's disease (CD) patients on maintenance treatment with anti-TNF agents. After two years of biologic treatment, transmural healing (TH) was achieved in about 25% of patients. Diagnostic criteria for TH were a bowel wall thickening (BWT) ≤3 mm using bowel sonography and a BWT ≤3 mm without signs of hypervascularization using magnetic resonance enterography. It should be noted that this study focused on mural findings rather than on mesenteric signs of inflammation.

Herein, we report an interim analysis of our prospective tertiary center study aiming to evaluate the correlation between endoscopic disease activity, fecal markers and Computed Tomography Enterography (CTE) findings of inflammatory activity at diagnosis and one year after initiation of immunosuppressive therapy. Consecutive patients with newly diagnosed CD were evaluated by endoscopy, CTE and fecal calprotectin at diagnosis and 12 months after beginning immunosuppression. Endoscopic severity was assessed using the simplified endoscopic score for Crohnís disease (SES-CD). Biomarkers, clinical indexes, and FC were recorded on the day of ileocolonoscopy at diagnosis and one-year after diagnosis. Radiological signs of inflammation evaluated were: mural thickness, mural hyperenhancement, mesenteric fat proliferation, mesenteric fat density, comb sign, presence of strictures, fistulas, abscesses, ascites and lymphadenopathy. These findings were evaluated in 5 predefined ileocolonic segments such as SES-CD, and each variable was scored as either 0 (absent) or 1 (present).

A total of 29 newly diagnosed CD patients have been enrolled [48% women, median age at diagnosis 30.0 (24.5–35.5) years]. At diagnosis, nearly all patients (n = 28, 97%) were aged between 17 and 40 years-old. Nineteen (65.6%) patients had exclusively ileal involvement (L1) and 14 (48%) had non-stricturing non-penetrating behaviour (B1). All patients had clinical and endoscopic active disease at baseline, with a median Harvey-Bradshaw Index, FC and SES-CD score of of 7.0 (6.0–9.0), 986.5 (361.8–3175.8) and 10.0 (7.0–16.0), respectively. At baseline all patients presented BWT and 97% presented mural hyperenhancement. Twenty-one (72%) patients presented increased fat density and hypervascularity and 18 (62%) strictures of at least one bowel segment (Table 1). Twenty-eight (97%) of patients were started on azathioprine, while 59% (n = 17) started biologic therapy (infliximab 11; adalimumab 6). At one-year follow-up, 24 (83%) patients were in clinical remission and 19 (66%) patients were in endoscopic remission. In contrast to Castiglione findings, in our cohort there was not a significant improvement in BWT at one year follow-up (Table 1). Indeed, endoscopic remission at one-year follow up only significantly correlated with improvement in mural hyperenhancement ($p = 0.004$), mesenteric fat density ($p = 0.001$), comb's sign ($p = 0.004$), and strictures ($p = 0.008$) (Table 1). It is known that at least some of the mural thickening observed despite endoscopic remission may not be due to active inflammation, but rather histological alterations secondary to transmural healing and it has been suggested that mesenteric findings may be better predictors of active disease [2–4]. According to that, our findings also suggest that an accurate diagnosis of transmural healing should be made based not only on mural findings but also on mesenteric signs of inflammation.

Table 1
Changes in CTE findings between baseline and 1 year of follow-up considering endoscopic activity.

	All patients (n = 29)			Endoscopic remission (n = 19)			Endoscopic activity (n = 10)		
	Baseline	1 year of FU	p-Value	Baseline	1 year of FU	p-Value	Baseline	1 year of FU	p-Value
Mural thickness	100% (n = 29)	79.3% (n = 23)	–	100% (n = 19)	68.4% (n = 13)	–	100% (n = 10)	100% (n = 10)	1
Mural hyperenhancement	**96.6% (n = 28)**	**62.1% (n = 18)**	**0.002**	**94.7% (n = 18)**	**47.4% (n = 9)**	**0.004**	100% (n = 10)	100% (n = 10)	1
Mesenteric fat proliferation	65.5% (n = 19)	58.6% (n = 17)	0.687	57.9% (n = 11)	47.4% (n = 9)	0.687	80% (n = 8)	80% (n = 8)	1
Mesenteric fat densification	**72.4% (n = 21)**	**20.7% (n = 6)**	**<0.001**	**68.4% (n = 13)**	**10.5% (n = 2)**	**0.001**	80% (n = 8)	40% (n = 4)	0.219
Comb's sign	**72.4% (n = 21)**	**34.5% (n = 10)**	**0.001**	**63.2% (n = 12)**	**15.8% (n = 3)**	**0.004**	90% (n = 9)	70% (n = 7)	0.5
Strictures	**62.1% (n = 18)**	**31% (n = 9)**	**0.004**	**57.9% (n = 11)**	**15.8% (n = 3)**	**0.008**	70% (n = 7)	60% (n = 6)	1
Lymphadenopathy	27.6% (n = 8)	13.8% (n = 4)	0.125	10.5% (n = 2)	0% (n = 0)	–	60% (n = 6)	40% (n = 4)	0.5
Fistulas	**31% (n = 9)**	**10.3% (n = 3)**	**0.031**	31.6% (n = 6)	10.5% (n = 1)	0.125	30% (n = 3)	10% (n = 1)	0.5
Ascites	10.3% (n = 3)	0% (n = 0)	–	5.3% (n = 1)	0% (n = 0)	–	20% (n = 2)	0% (n = 0)	–
Abscesses	10.3% (n = 3)	0% (n = 0)	–	5.3% (n = 1)	0% (n = 0)	–	20% (n = 2)	0% (n = 0)	–

p < 0.05.

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Conflict of interest statement
None declared.

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Transmural healing in Crohn's disease: Beyond mural findings – Authors' reply

Dear Editor,

We sincerely appreciated the interest and the comments on our paper by Lopes et al. [1,2]. The authors refer about their interesting study including 29 newly diagnosed patients with active Crohn's disease (CD), all performing endoscopy and computed tomography enterography (CTE). After one year of follow-up, 6 out of 29 patients (20.7%) achieved the transmural healing (TH). Particularly, the authors put their attention on a new, interesting but unexplored, endpoint: the "extramural healing", defined as the radiological remission of all signs of inflammation beyond mural findings (e.g. increased mesenteric fat density, mesenteric fat proliferation, comb's sign, lymphadenopathies). In effect, Lopes et al. found that endoscopic remission at one-year follow-up significantly correlated only with improvement in mural hyperenhancement ($p = 0.004$), mesenteric fat densification ($p = 0.001$), comb's sign ($p = 0.004$), and strictures ($p = 0.008$), but they did not find any significant improvement in bowel wall thickness (BWT). However, it is important to note that in their study no statistically significant improvement was observed either in mesenteric fat proliferation ($p = 0.6$) or in presence/features of lymphadenopathy ($p = 0.1$), that are considered two important signs of active inflammation.

Moreover, it appears quite difficult to compare our results [2] to those by Lopes et al. [1]: in effect, we enrolled only patients who completed a 2-years period of treatment with anti-TNF, while their study ended at one year; this aspect could justify the lower rate of TH obtained in their series (20.7%). Furthermore, only 17 subjects (59%) in Lopes et al. series started an anti-TNF treatment, while the remaining CD population was put on thiopurines. About this topic, we previously reported [3] that TH can be achieved after two years in 25% of patients treated with anti-TNF and in only 4% of subjects treated with thiopurines ($p = 0.01$; OR 6.2), thus concluding that only biologics are able to achieve this strong outcome. It would be interesting to know if patients who achieved TH at one year in the study by Lopes et al. [1] were on anti-TNF or on thiopurines.

A recent study by Fernandes et al. [4] reported that patients presenting TH (defined as normalization of BWT) had lower rates of hospitalization, surgery, and therapy escalation than patients with only mucosal healing or no healing. Interestingly, comb's sign was used as additional feature for assessment of disease activity only in inconclusive cases.

Deepak et al. [5] reported that radiological response (using MRI or CTE) was highly predictive of reductions in long-term risk of hospitalization, surgery, or steroids use among patients with small bowel CD. Remarkably, they considered also comb's sign and mesenteric edema in their radiological protocol. However, the most impressive outcomes were correlated to BWT and bowel enhancement.

In conclusion, we agree with Lopes et al. that the evaluation of extramural signs of inflammation (by MRI, CTE or ultrasonography) should be carefully evaluated and interpreted by radiologists/gastroenterologists. At the same time, further studies are needed in order to evaluate the prognostic role of residual mesenteric findings in patients achieving TH.

We strongly encourage further studies, like that by Lopes et al., attempting to evaluate, characterize and better define the concept of "transmural healing" as new promising therapeutic target in CD.

Guarantor of article

Prof Fabiana Castiglione.

Conflict of interest
None declared.

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ORIGINAL ARTICLE

Observational Study

Capsule enteroscopy is useful for the therapeutic management of Crohn's disease

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Author contributions: Santos-Antunes J wrote the paper, collected the data, elaborated the database, analyzed the statistics, and contributed to the interpretation and literature review; Cardoso H contributed to capsule reading, data collection, and study design; Lopes S contributed to patient follow-up and scientific collaboration; Marques M contributed to capsule reading and scientific collaboration; Nunes ACR contributed to patient follow-up and scientific collaboration; Macedo G contributed to study design, paper writing, and scientific revision of the manuscript.

Institutional review board statement: Due to its retrospective nature, no ethical concerns were raised for the writing of this manuscript.

Informed consent statement: Informed consent for CE and Patency capsule procedures was obtained for every patient. Due to its retrospective nature, no informed consent for the writing of this manuscript was applicable.

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Abstract

AIM: To analyze therapeutic changes in Crohn's disease (CD) patients following video capsule endoscopy (VCE) and to assess the usefulness of Lewis score and the Patency Capsule.

METHODS: Patency Capsule was performed in every patient that had indication for VCE, and those with negative patency did not undergo VCE. Patients with established CD that underwent VCE between January 2011 and February 2014 were selected for this study; those with suspected CD were excluded, independent of VCE results, since our purpose was to address differences in therapeutic regimen in CD patients before and after VCE. Patients with inconclusive VCE were also excluded. Patients had to be free of non-steroidal anti-inflammatories for at least 1 mo. Those patients who met these criteria were allocated into one of three groups: Staging group (asymptomatic CD patients that underwent VCE for staging of CD), Flare group (patients with active CD), or Post-op group (CD patients evaluated for post-operative recurrence). Lewis score was calculated for every VCE procedure. Statistical

analysis was performed to address the impact of VCE findings on the therapeutic management of CD patients and to evaluate the utility of the Lewis score.

RESULTS: From a total of 542 VCEs, 135 were performed in patients with CD. Patency capsule excluded nearly 25% of the patients who were supposed to undergo VCE. No videocapsule retention during VCE was reported. From these 135 patients, 29 were excluded because CD diagnosis was not established at the time of VCE. Therefore, a total of 106 patients were included in the final analysis. From these, the majority were in the Staging group (n = 73, 69%), and the remaining were in the Flare (n = 23, 22%) or Post-op (n = 10, 9%) group. Median time between diagnosis and VCE was 5.5 years. Overall, VCE determined changes in the treatment of 40% of patients: only 21% remained free of immunosuppressors after VCE compared to 44% before VCE (P < 0.001). The differences in therapy before and after VCE achieved statistical significance in the Staging and Flare groups. In addition, patients were significantly different when stratified regarding time since diagnosis to the date of VCE. A higher Lewis score was associated with therapeutic modifications (P < 0.0001); where a score higher than 1354 was related to 90% probability of changing therapy [area under the receiver operative characteristic (AUROC) 0.80 (95%CI: 0.69-0.88)].

CONCLUSION: VCE significantly changed the therapeutic management of CD patients, even in those with long-term disease. Systematic use of Patency capsule allowed for no videocapsule retention.

Key words: Capsule enteroscopy; Crohn's disease; Treatment modification; Patency capsule; Lewis score

Core tip: Our work analyzed the therapeutic management of patients with Crohn's disease (CD) and concluded that a very significant proportion of patients modify their therapeutic regimens after performing video capsule endoscopy (VCE), even in those with long-term disease or those without symptoms. This finding highlights the importance of this procedure in the management of CD. The systematic use of Patency capsule is controversial; however, we showed in our study that after excluding patients with negative patency, who did not undergo VCE, none of the patients had video capsule retention during VCE, highlighting the importance of Patency capsule in this setting.

Santos-Antunes J, Cardoso H, Lopes S, Marques M, Nunes ACR, Macedo G. Capsule enteroscopy is useful for the therapeutic management of Crohn's disease. *World J Gastroenterol* 2015; 21(44): 12660-12666 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i44/12660.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i44.12660

INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory disease associated with mucosal and transmural inflammation of the bowel wall, and its diagnosis relies on the combination of clinical, endoscopic, radiologic and histopathological features.

Regarding endoscopic assessment, ileocolonoscopy is the first procedure for the establishment of the diagnosis. However, evaluation of the entire small bowel is mandatory, since it can change the therapeutic approach used and overall prognosis[1]. In this setting, international guidelines[1] regard cross-sectional studies, such as entero-computed tomography (CT) scan or magnetic resonance imaging (MRI), as first line tools, since they can evaluate extra-luminal features and characterize intra-abdominal adverse events related to CD, such as abscesses or fistulas. Video capsule endoscopy (VCE) is considered a second-line tool in those CD patients with atypical symptoms, in which imaging was negative[1].

Nevertheless, VCE is often used as a first-line study in the suspicion of CD, after ileocolonoscopy[2,3]. Its efficacy in detecting lesions in the upper small bowel seems higher than entero-CT or MRI, with similar accuracy for distal lesions[4-6].

Since the role of VCE in established CD is not completely defined, namely whether VCE is useful for treatment guidance, a few studies tried to evaluate its impact on determining treatment guidance and analyzing the therapeutic changes attributed to VCE[7-11]. However, some of these previous studies included a very low number of patients or had very short disease duration at the time of VCE, thereby compromising the interpretation of the results. Evaluation of possible changes in management includes searching for changes in inflammatory bowel disease (IBD) specific modification strategies, further radiologic or endoscopic studies, or even surgical interventions.

In this study, our main goal was to analyze the changes in the therapeutic regimen of patients with long-term CD after undergoing VCE. Additionally, we studied the impact of Lewis score in this setting and the number of videocapsule retentions with the systematic use of Patency capsule.

MATERIALS AND METHODS

All patients with established CD that underwent VCE since January 2011 to February 2014 were included in the study. Patients were assigned to one of three groups. The first group (Staging group) included patients with clinical remission who underwent VCE to assess disease extent or small bowel re-evaluation. The second group (Flare group) included patients who were undergoing re-evaluation because of a flare and had clinical deterioration or raised inflammatory markers. The third group (Post-op group) included

Table 1 Baseline characteristics of the patients included in the study *n* (%)

Population characteristics (*n* = 106)	Value
Male gender	47 (44)
Age - mean	40 ± 13 yr
Median time between diagnosis and VCE	5.5 (IQR 2-10) yr
Montreal classification	
Age at diagnosis	
A1: Below 17	7 (7)
A2: 17-40	84 (79)
A3: Above 40	15 (14)
Behavior	
B1: Non-stenosing/non-penetrating	86 (81)
B2: Stenosing	12 (11)
B3: Penetrating	8 (8)
Location	
L1: Terminal ileum	74 (70)
L2: Colonic	10 (9)
L3: Ileocolonic	22 (21)
+ L4: Upper disease	38 (36)
Treatment before VCE	
Anti-TNF	21 (20)
Immunosuppressors	38 (36)
Aminosalicylates only	40 (38)
No treatment	7 (6)

VCE: Video capsule endoscopy; TNF-α: Tumor necrosis factor α.

patients who were being evaluated for post-operative recurrence.

Patients with suspected CD in which VCE did not confirm the diagnosis or those with VCE considered inconclusive were excluded from the study. Also, patients with suspected CD in which VCE confirmed the diagnosis were also excluded, since our main goal was to evaluate changes in therapeutic regimens for CD before and after VCE. Patients had to be free of non-steroidal anti-inflammatories for at least 1 mo.

All the VCEs were performed after confirming small bowel patency using Agile Patency capsules (Given®, Imaging Ltd. Yoqneam, Israel), which were read 30 h after ingestion. VCEs were performed using PillCam® SB2 or SB3 capsules (Given®, Imaging Ltd.). On the previous day, patients were asked to follow a liquid diet and to perform a bowel preparation. On the day of the procedure, patients were on a clear-liquid diet for 6 h after swallowing the capsule. RAPID® Real-Time Viewer was performed in all patients after 2 h of ingestion, and domperidone 10 mg was prescribed if the capsule remained in the stomach. A new evaluation was performed 1 h later, and if the capsule was still retained in the stomach, a new dose of domperidone 10 mg was administered. If the medication failed, an upper endoscopy was performed to place the device in the duodenum.

All the exams were read by two experienced gastroenterologists using RAPID Reader®. Lewis score was calculated in order to assess the severity of the disease in all procedures, being classified as normal or clinical insignificant if lower than 135 points, mild disease between 135 and 790, and moderate/severe disease above 790, as described elsewhere[12].

In addition to demographic, clinical, and analytical data, medical therapy at the time of VCE and therapy modifications due to VCE were recorded. In order to simplify the results, the "Anti-tumor necrosis factor (TNF) group" included patients taking anti-TNF (infliximab or adalimumab) in monotherapy or combination therapy, and the "Immunosuppression group" included those under azathioprine (AZA) either in monotherapy or combination with 5-amynosalicilates (5-ASA); the remaining patients were under monotherapy with 5-ASA or had no therapy.

Changes in CD treatment in the Staging group and Post-op group were only attributable to VCE findings, since these patients were in clinical and analytical remission. Patients in the Flare group had clinical or analytical active disease, but statistical analysis was conducted to conclude if VCE findings were associated with changes in therapeutic regimen, independent of the flare itself.

Statistical analysis was performed using SPSS software version 22 (SPSS Inc., Chicago, IL, United States). Continuous variables were analyzed using T-student tests or Mann-Whitney test when normal distribution was not verified. Categorical variables were analyzed using Pearson's Chi-square, Fisher's exact tests or McNemar test as appropriate. Logistic regression was performed in order to assess variables independently associated with changes in therapeutic regimen. A *P* value below 0.05 was considered statistically significant.

RESULTS

Among the 542 VCEs performed during the analyzed period, 135 were performed in patients with CD, after positive patency was confirmed by Patency capsule (Patency capsule excluded nearly 25% of the patients who were supposed to perform VCE). From these 135 patients, 29 were excluded because they did not have established diagnosis of CD at the time of VCE. In total, 106 patients were included for the final analysis.

Most of the procedures were performed in patients within the Staging group (*n* = 73, 69%), with the remaining patients in the Flare (*n* = 23, 22%) and Post-op (*n* = 10, 9%) groups. Baseline characteristics are shown in Tables 1 and 2. Fifty-six percent were female, with mean age of 40 ± 13 years. Most patients (81%) had an inflammatory phenotype; 70% had isolated ileal disease. After VCE analysis, upper tract involvement was identified in 49 (46%) patients.

The median time between the diagnosis of CD and VCE was 5.5 [interquartil range (IQR) 2-10] years. Regarding disease activity (Lewis score), 51 (48%) had normal or clinical insignificant lesions (25% of the total procedures were normal), 14 (13%) had mild disease, and 41 (39%) had moderate to severe disease.

Table 2 Patient characteristics at baseline that could influence therapeutic changes after video capsule endoscopy

Variable	Univariate analysis	Multivariate analysis
Age	$P = 0.477$	-
Male gender	$P = 0.517$	-
Smoking	$P = 0.771$	-
C-reactive protein	$P = 0.188$	-
Disease time duration	$P = 0.073$	-
Age at diagnosis	$P = 0.097$	-
Ileal *vs* colonic *vs* ileocolonic disease	$P = 0.009$	$P = 0.367$
Disease behaviour	$P = 0.564$	-

Overall, VCE results guided changes in the treatment of 40% of the patients. At the time of VCE, 38% were under 5-ASA, 36% were under immunosuppressors, 20% were under Anti-TNF, and 6% had no treatment. After VCE, no patient remained without therapy; and the percentage of patients under 5-ASA decreased to almost half and those under AZA and anti-TNF rose significantly ($P < 0.0001$, Figure 1). Similarly, these results were significant when stratifying patients based on time between diagnosis and VCE (less than 1 year and more than 1, 5, and 10 years of the disease) (data not shown). Overall, only 21% of the patients remained free of immunosuppressors after VCE compared to 44% before VCE ($P < 0.001$).

When analyzing Lewis score, only 7% with normal or almost normal VCE changed therapy, whereas 67% changed therapy when VCE demonstrated moderate to severe disease ($P < 0.0001$, Figure 2). Those patients who changed therapy clearly had higher median Lewis score values (1446 *vs* 552, $P = 0.006$). Patients with a Lewis score higher than 1354 had a 90% probability of changing their medication [AUROC 0.80 (95%CI: 0.69-0.88)].

We found differences in the median Lewis score among the different groups. Patients in the Flare group had higher Lewis score values than the Staging (1648 *vs* 816, $P = 0.040$) and Post-Op (1648 *vs* 327, $P = 0.035$) groups. No significant differences were found between Staging and Post-op groups.

Regarding the indication for VCE, the percentage of patients under AZA was duplicated in the Staging group ($P < 0.0001$) after VCE, while in the Flare group, the number of patients doubled under anti-TNF ($P = 0.032$). In the Post-op group, there was an increase in the number of those taking AZA or anti-TNF, but it was not statistically different ($P = 0.133$, Figure 3).

When performing multivariate analysis, we found that the factors age, C-Reactive protein levels, smoking habits, or duration of the disease were not linked with changes in therapeutic regimen after VCE.

VCE was not retained in any of the patients. Furthermore, no clinical symptoms or other adverse events were reported in the patients where Patency capsule demonstrated negative patency.

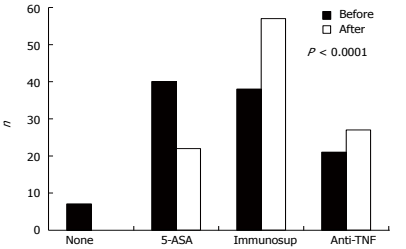

Figure 1 Therapeutic regimens for Crohn's disease before and after capsule enteroscopy. TNF: Tumor necrosis factor; 5-ASA: 5-aminosalicylic acid.

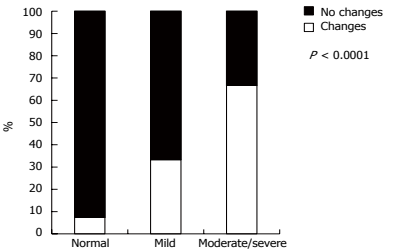

Figure 2 Changes in therapeutic regimen regarding the Lewis score calculated with video capsule endoscopy.

DISCUSSION

VCE is a valuable tool in the assessment of the small bowel[13-19], but its importance in the evaluation and follow-up of CD patients is not well established[20,21]. An indicator to determine the impact of this method on CD is modification of the therapeutic approach after VCE. Some studies address this issue, but there were some limitations.

A recent study[8] found that the number of patients with CD under anti-TNF or immunosuppressants rose after VCE. However, most of the patients underwent VCE in the first year of the disease; and, consequently, 48 of 50 patients were only on 5-ASA or steroids before VCE (only one was under AZA and one under anti-TNF), which makes this difference expectable. An advantage of our study is the inclusion of patients with long-term disease (median time 5.5 years) and patients whose CD is adequately managed, namely anti-TNF and immunosuppressants. Our results showed that VCE was decisive for therapeutic changes even in patients with more than 10 years of CD evolution,

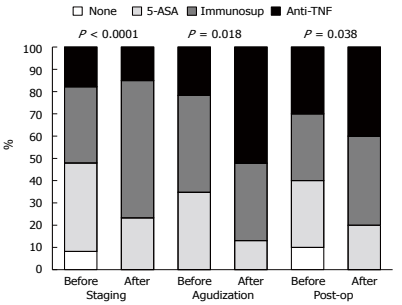

Figure 3 Therapeutic regimens before and after capsule enterosopy within the three analyzed groups. TNF: Tumor necrosis factor; 5-ASA: 5-aminosalicylic acid.

highlighting the importance of VCE in this pathology. Similar results were found in a previous study of 71 patients with CD that included subjects with long-term disease[10]. The treatment modification rate was even higher in a study performed in a pediatric population with CD[22].

VCE promoted changes in therapy in every group, although in the Post-op group a statistically significant difference was not achieved, probably due to the small amount of patients (n = 10). In fact, post-op evaluation is emerging as a potential indicator for VCE[23,24]. It is considered to have the same sensibility, specificity, and negative and positive predictive values[2] as colonoscopy. In addition to the latter being a more accessible procedure, VCE was previously shown to find more endoscopic recurrences than colonoscopy[25], with the advantage of allowing for proximal small bowel evaluation[26].

In the Staging and Flare groups, there was a clear and significant modification in therapeutic regimens after VCE, with a decrease in the number of patients under 5-ASA and an increase in patients under AZA (Staging group) and anti-TNF (Flare group). Downgrading of therapy was observed in three patients (infliximab to AZA), all in the Staging group. Those were patients with long-term remission who were under combination therapy and, after performing a VCE for address the possibility of anti-TNF withdrawal, had a normal exam. Previous studies have determined the utility of VCE for the assessment of small bowel mucosal healing after immunomodulator or biologic therapy[27,28], eventually contributing to a downgrade in CD therapy.

It should be noted that none of our patients were being treated with steroids. This was due to the duration of time between the flare and the realization of VCE. Patients in the Flare group started steroids as indicated, but when they came to undergo VCE, the steroid cycle was already completed. This delay in VCE could have been a problem in our analysis since some patients may escalate therapy upon flare before VCE, which could contribute to some attenuation in the differences between therapy before or after the VCE. Statistical differences were still found despite this time lag, making changes in therapy regimens more attributable to VCE findings than to clinical or analytical flare.

The importance of the VCE *per se* in the changes in therapeutic regimens was highlighted by the regression analysis. As observed, no other factor presented at baseline was independently related to therapeutic modifications. Age, gender, smoking habits, duration of the disease, and inflammatory markers at the time of the VCE were not determinant for therapeutic decisions, making therapeutic changes attributable to the results of VCE. Previous studies had already shown a weak correlation between VCE results and inflammatory biomarkers, making VCE very useful even in the absence of raised C-reactive protein or fecal calprotectin[11].

Lewis score can be a valuable tool for therapeutic management[29]; as expected, higher scores were related with more frequent changes in medical therapy, since they represent active disease requiring a more aggressive treatment.

All the VCEs were performed after confirming small bowel patency using Agile Patency capsules. This device proved to be a very useful tool for patients with known stenosis[30], but its systematic use, as we perform in our institution, is not consensual. International guidelines[2] state that the risk of capsule retention is high in patients with known CD, and, therefore, patency capsule or cross-sectional studies must be performed before VCE to exclude significant stenosis. In patients with suspected CD, the risk appears to be much less significant, and its use is controversial. Since patients with CD can have inflammatory changes in small bowel mucosa, raising the risk of capsule retention, we performed Patency capsule in every patient in this setting. In our Department, nearly 25% of the patients with CD do not perform videocapsule due to negative patency as assessed by Patency capsules. Consequently, we did not experience any videocapsule retention during VCE.

The main limitations of our study were the small number of patients in the Post-Group, which precluded significant results (although there was a clear trend towards therapeutic modifications after VCE), and its retrospective nature. Since this work was not designed to compare patients with and without VCE, we did not assess the differences in the follow-up between them. However, it is well known in the literature that a suboptimal treatment of CD could predispose to a worse outcome. Therefore, we strongly believe that therapy escalation, even in patients with clinical remission but with small bowel lesions detected by VCE, is of paramount importance for a better long-

term outcome of CD.

Overall, we concluded that VCE is a very powerful tool for evaluating CD in all groups of patients, including those with long-term disease under immunosuppressors and anti-TNF. It was decisive for treatment guidance, which ultimately can lead to an earlier introduction of immunosuppressors and anti-TNF therapy, consequently improving overall prognosis.

COMMENTS

Background

The role of video capsule endoscopy (VCE) in treatment guidance is not well established for Crohn's disease (CD). Previous studies have attempted to address this subject, but the data are still scarce, especially in patients with long-term disease.

Research frontiers

The authors' results demonstrated the utility of VCE and Patency capsule for the management of CD, namely for the guidance of medical therapy.

Innovations and breakthroughs

This study included patients with long-term CD and show how VCE can affect medical therapy. In addition, the clinical utility of patency capsule is well documented.

Applications

Patency capsule allowed for no VCE retention. A large proportion of patients with CD changed therapeutic regimen after VCE.

Terminology

Lewis score classification according to VCE findings: normal or clinical insignificant disease if lower than 135 points, mild disease between 135-790, and moderate/severe disease above 790 points.

Peer-review

The authors have reported the usefulness of VCE for the management of the therapeutic regimen in patients with CD. This manuscript is well-written.

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Discussion

In the Results section we have included the research articles relevant for this thesis. In this Discussion section, the main results of our work will be further dissected.

Virus and IBD

Inflammatory Bowel Disease has been gaining increasing importance and interest in the Gastroenterological milieu over the last two decades. This is mainly due to 5 reasons: 1. an increase in disease prevalence; 2. the affection of a young population, leading to a great impairment in quality of life and work productivity; 3. better accessibility to small bowel disease, enabling to monitor disease activity with non-invasive methods; 4. the amount of recent knowledge related to disease immunopathogenesis; 5. the evolving therapeutic options, with more efficacious drugs, able to potentially modify favorably the disease course.

Despite all these improvements in disease pathogenesis, diagnosis and management, a full comprehension of its cause is still lacking. The recognition of the importance of microbiome in different digestive diseases raised, once more, the interest in a potential role of an infectious agent in disease etiology/course. The gut virome, the viral component of microbiome, outnumbers the gut bacterial population. It has been demonstrated its potential to alter host physiology and homeostasis, and its change in composition throughout an individual´s life[8]. It has also been speculated that the interaction of virus and commensal microbiome, susceptibility genes and immune system may be responsible for patient´s clinical heterogeneity and variability in response to different treatments. Several works tried to link viral dysbiosis with IBD pathogenesis without consistent results.

An alternative line of interest is the possible influence of viral reactivation, during immunosupressive states, in disease course and response to therapy. Data from the transplant setting have highlighted the influence of reactivation of virus of the Human Herpesviridae family (CMV and EBV) in disease course and long term prognosis. In literature there is conflicting evidence of the implications of viral reactivation in IBD. While some

authors suggest that CMV reactivation is more common in UC and is associated with disease complications and loss of response to immunosupressors, others state that viral load does not impact disease outcome[26, 33, 180, 181]. On the other hand, the focus on EBV reactivation in IBD patients has been centered in the risk of malignancy under immunosuppression, especially in young male patients. Little is known about its prevalence in IBD patients, its role in disease course and response to therapy and the real influence of immunosupressors and biologics on viral load and development of lymphoproliferative disorders in this context. Another DNA virus of the Herpesviridae family that has been implicated in gastrointestinal symptoms in immunosupressed patients is HHV-6. In IBD, HHV-6 was found to be more prevalent in serum and associated with more severe endoscopic lesions[50].

One major challenge when evaluating the prevalence and influence of these opportunistic infections on disease course is the distinction between a superimposed infection and a disease relapse. Recent data highlighted the interest of quantifying viral load by PCR in order to establish proper diagnosis and management[29, 42, 182].

A previous work from our group[43] has demonstrated that IBD patients, irrespective of treatment, have a higher prevalence of EBV in blood compared to controls. In that cohort, treatment with infliximab and older age were risk factors for EBV prevalence. When that population positive for EBV was stratified by the number of EBV copies/ml, no differences were found with respect to the type of treatment, and no clinical consequences were found.

In our study comparing 95 IBD patients with 50 healthy controls, accordingly to previous papers, we found a higher prevalence of EBV and CMV in the mucosa of the disease group. Despite the more commonly reported association between CMV and UC, we did not find any difference in the prevalence of CMV or EBV in the mucosa of CD and UC patients. As we hypothesized, we found a higher prevalence of both EBV and CMV in areas with active endoscopic disease compared to areas of normal mucosa (although these results were only significant for EBV in both CD and UC). We also tried to determine if viral load had any influence in disease severity and prognosis, trying to define a cut-off value with therapeutic/management implications.

[180] Yoshino T *et al.* Inflammatory Bowel Diseases. 2007.

[181] Ganzenmueller T *et al.* Journal of Clinical Virology: the Official Publication of the Pan American Society for Clinical Virology. 2009.

[182] Ciccocioppo R *et al.* Immunologic Research. 2016.

Comparing median viral loads (copies/105 cells) of CMV, EBV and HHV6 in ulcerated and normal mucosa, on both CD and UC, we did not find any statistical difference, although we found a higher EBV median viral load in inflamed mucosa of CD patients. Comparing viral load in UC and CD mucosa, we found a higher median EBV viral load in inflamed mucosa of CD patients compared with inflamed mucosa of UC patients (p=0.010). The same was not true with respect to CMV or HHV6. Despite not statistical significant, CMV median viral load was higher in ulcerated mucosa of UC patients (p=.429). In this study, we were not able to establish a correlation between viral load and endoscopic disease activity. In contrast to what our group previously found[43], in this cohort we did not find a difference in viral serum prevalence between patients and controls nor between different treatment regimens. One possible explanation may be the fact that we included patients with active disease while on the previous work only patients in remission were analyzed. In addition, the mean age of our control group was higher than that published in 2013. In line with our findings, there are studies suggesting that only steroids and anti-TNF-alpha agents are associated with EBV colitis, and not the use of immunossupressors or the duration of therapy[42, 183].

In summary, we were able to confirm a higher prevalence of EBV, and to a lesser extent of CMV, in the mucosa of IBD patients compared to healthy controls. According to other reported results[28, 37], we also found EBV as the most prevalent agent, with a higher prevalence of viral DNA in the mucosa compared to peripheral blood. This may suggest a viral potential role in the immune disturbance present on IBD patients´ intestinal mucosa, with implication in disease pathogenesis, rather than disease severity. Other findings supporting the hypothesis of EBV triggering role was the observation of a similar prevalence both in inflamed and non-inflamed mucosa. Another supporting factor was the absence of correlation between mucosal viral load and severity of endoscopic activity. Even in normal mucosa, IBD patients had a higher prevalence of EBV compared to healthy controls, although there were no significant differences in the median viral DNA levels.

[183] Ford AC *et al.* The American Journal of Gastroenterology. 2013.

As CMV was inexistent in normal mucosa, and with very low viral load in inflammed mucosa, no conclusion could be drawn regarding CMV infection. Regarding HHV6, and concordant with other works[49], it seems to have no role in IBD pathogenesis, as its prevalence and viral load was similar between the 2 groups, suggesting that not all virus of the Herpesviridae family are associated with IBD pathogenesis.

We did not find any major advantage of DNA quantification as we could not establish a correlation between endoscopic disease severity and viral load. Nevertheless, a note of caution should be made when trying to extrapolate these findings to the general IBD population, as this cohort includes only patients with endoscopically active disease and a high proportion of subjects under immunosuppressors.

Fecal Markers and Disease Recurrence

Despite the remaining uncertainties concerning disease etiology and the role of infectious agents, our knowledge and attitude in disease management has evolved enormously in the last decades. As the primary therapeutic goal of CD has shifted from clinical remission to achieving mucosal healing, it seems more and more important to objectively access the response to therapy. As symptoms and serum biomarkers have proved not to be good surrogate markers of mucosal healing, attention has shifted to endoscopy and fecal markers. In the context of postoperative follow-up of CD patients, there is enough evidence that endoscopic recurrence, irrespective of the presence of clinical recurrence, predicts the clinical course. It has also been shown the short-term benefits of postoperative endoscopic evaluation and early treatment intensification in recurrent disease.

All papers published to date concerning post-operative CD, use the Rutgeerts Score to classify endoscopic recurrence. However, this score is still not validated, lacks inter-observer agreement and gathers in the same category patients with lesions limited to the anastomosis and patients with new onset ileitis. The Modified Score considers

recurrent disease only if lesions develop in the ileum, with strictures and ulceration of the anastomosis being considered non-recurrent disease. The use of this Modified Score as not been generalized, although to our group it conceptually seems more appropriate. In recent years, the use of fecal markers in this context has gained a lot of attention, due to its noninvasiveness and possibility of being repeated as needed. The majority of the initial studies enrolled small number of patients and reached conflicting results[165,167-173]. A landmark in this subject was the study of Wright *et al*[167] that evidenced the predictive value of serial monitoring of FC after surgery for identifying patients likely to relapse and performing early endoscopic evaluation in order to intensify therapy if recurrence was diagnosed.

In our cohort of 99 patients previously submitted to resection, 91% were in clinical remission at the time of colonoscopy. Despite that, 34% presented endoscopic recurrence with median values of fecal markers significantly higher than the group of patients with no recurrent disease. The calculated best cut-off value for predicting recurrence for FC and FL was 100 ug/g and 7.25 ug/g, with NPV of 91% and 90% respectively. The AUROC for FC was 0.831 (95% CI, 0.752-0.911; $p<0.05$) and for FL was 0.842 (95% CI, 0.763-0.920; $p<0.05$). This threshold, as a screening test, reassures that the vast majority of patients with lower values will be in remission and will not be missed for subsequent colonoscopy. FL performed equally well in the diagnosis of recurrence, but we could not confirm an improvement in diagnostic accuracy by combining both markers. Using either FC or FL is sensitive enough to diagnose recurrence, and preferring one over the other depends on personal experience and local availability. As it would be expected, if the Rutgeerts score was used more patients were diagnosed as having endoscopic recurrence, and the median values of both FC and FL were lower than with the Modified Score, reflecting the group of patients with normal FC and FL and only anastomotic disease. Using the Rutgeerts Score the performance of both tests would have been extremely jeopardized with a significant decrease in both accuracy (FC 55% and FL 60%) and NPV (FC 33% and FL 37%).

Our findings, in accordance to previous papers, suggest that both FC and FL are sensitive enough to monitor CD recurrence postoperatively,

reassuring both clinicians and patients that few endoscopic recurrences will be missed using these noninvasive tests. We were able to define cut-off values for both markers, with similar results to previous papers [70]. An innovative finding was the use of Modified Rutgeerts Score, and the reinforcement that the i2 subgroup should be divided in i2a and i2b, considering only the latter as endoscopic recurrence.

In the other study we intended to demonstrate the accuracy of FC and FL in diagnosing disease recurrence in the context of anastomotic stricture, and the efficacy and safety of EBD in this context. We evaluated 48 patients with anastomotic strictures and, in this cohort, both FC and FL were able to predict disease recurrence guiding the need for EBD. In this group of patients only 17% presented subocclusive symptoms and no serum biomarker correlated with endoscopic or radiographic findings. We demonstrated that patients with an asymptomatic anastomotic stricture and a low value of both FC and FL, have a high probability of being in remission. On the other hand, patients with high levels of fecal markers, have a high probability of recurrence, not suspected by clinical or serum markers. These patients should have the anastomosis dilated in order to evaluate the proximal mucosa allowing the optimization of therapy accordingly. We diagnosed recurrence in 22/48 patients (16 with severe disease) only after EBD. In this cohort, the best calculated cut-off values for both FC and FL to predict recurrence were 90.85 ug/g and 5.6 ug/g, respectively. This is in accordance with other published studies that define in the postoperative setting a cut-off value higher than the 50 ug/g used to diagnose IBD.
These two works also used the Modified Rutgeerts Score for the first time in comparison to fecal markers. The authors believe that the presence of lesions limited to the anastomosis should not define recurrent disease as many other conditions may be responsible for that finding. We also demonstrated that this modified score has a better correlation with fecal markers in recurrence diagnosis, although these results need to be validated and reproduced by other groups.

Endoscopic Balloon Dilation

Despite being controversial, we believe that strictures should be dilated, irrespective of symptoms, in order to evaluate the proximal mucosa and treat to target. In our pool of 162 CD patients submitted to ileocecal resection/right hemicolectomy, evaluated by colonoscopy and with a median follow-up period of 4.4 years, a third of patients presented an anastomotic stricture. EBD was performed in 26.5%of patients, with a technical success rate of 97.7%, no major adverse events and a long-term efficacy of 37%. This high success rate is in accordance to what is already published[97, 99], and the need to repeat dilation did not reduce the procedure efficacy.

Therapeutic changes were made after dilation in more than a third of patients, due to recurrence diagnosis. With this strategy surgery may be delayed or even avoided in a large number of patients, by stricture resolution and by preventing disease progression (therapeutic intensification after dilation if needed). Only 2 patients required surgery after dilation, due to symptoms worsening. We were not able to find any predictive factor of subsequent dilation. Contrary to some data in the literature[63, 184], smoking, therapy at the time of dilation, endoscopic disease activity or biomarkers were not found to influence the need to repeat dilation.

The results of these works, emphasize the need of more sensitive and specific markers of disease recurrence, since the majority of patients were in clinical remission with normal serum biomarkers. Our results reinforce the believe that fecal biomarkers should be incorporated in the postoperative management algorithm, both to diagnose recurrence and to assess response to therapy. In patients with low levels of FC (<100 ug/g) and/or FL (<7.25ug/g), in clinical remission, endoscopy may be postponed, while in patients with high values of fecal markers, endoscopy should be performed in order to diagnose disease progression and prompt escalate therapy if needed.
We also demonstrated that EBD is a safe and effective alternative to surgery with good short and long-term outcome. Our group has been observing that dilating strictures irrespective of symptoms, has an impact on patients´ management and disease course, allowing evaluation of disease activity and therapeutic adjustments.

[184] Ding NS *et al*. Journal of Crohn's & Colitis. 2016.

Radiology, Fecal Markers, VCE and Disease Activity

Despite being considered the gold standard to evaluate disease activity and response to therapy, in CD ileocolonoscopy alone may be insufficient to assess disease location and extension. Cross sectional imaging has emerged as the preferred modality in small bowel evaluation, both to report disease activity and complications, and to monitor response to therapy. As it has been reported a radiological response to anti-TNF alpha agents[125] predicting lower rates of hospitalization, surgery and steroids use in the long-term, maybe transmural healing will surpass mucosal healing as the therapeutic target in CD.

As ileocolonoscopy is invasive and only evaluates the mucosa, we planned to evaluate how well CTE findings and FC correlate with endoscopic disease activity. In our prospective cohort of 29 patients, we found a positive correlation between endoscopic and radiological findings. Some studies have shown a correlation between disease activity and mesenteric signs of inflammation[165, 167, 168]. With that in mind we adapted a CTE scoring system that included mesenteric variables such as fat densification and proliferation and the comb sign, in addition to mural thickening, as signs of disease activity.
We were able to establish a strong correlation between endoscopic activity defined by SES-CD and mesenteric findings. In patients with endoscopic remission at one-year, we found a significant improvement in bowel wall hyperenhancement, mesenteric fat densification and comb sign with a significative decrease of the median CTE score. This was in accordance with previous reports[120, 125]. We also demonstrated a correlation between FC, endoscopic score and CTE signs of inflammation. A cut-off value of 100ug/g for FC predicted endoscopic remission with a NPV of 81% and an accuracy of 83%. Although there are currently alternative diagnostic modalities radiation-free, CTE still is the most widely available modality, is cheaper, faster, with the best spatial resolution, with no individual limitations and very well tolerated by patients.

With this work we suggest that both radiological evaluation and FC may be used as alternatives to endoscopy in monitoring disease response to therapy. Besides mural findings, mesenteric markers of inflammation should also be looked for when evaluating disease activity. We also believe that similarly to MRE, where several scores have been developed, a CTE score of disease activity should be developed and validated. In this group, a CTE score ≤ 3 points and a FC < 100ug/g accurately predicted endoscopic remission at one year follow-up.

Considering other noninvasive method of evaluation of the small bowel, VCE is gaining importance and utility as a tool for treatment guidance. Knowing that CD is a silent disease, it is reasonable to hypothesize that even in patients with long duration compensated disease mucosal lesions may be present. Although data available in the literature is scarse, there have been some reports of treatment modifications in CD patients after VCE[82, 83, 86, 185-187] and evidence that mucosal healing assessed by VCE is associated with clinical remission in the short and long-term[85, 188]. In our experience, the performance of VCE in asymptomatic patients with long disease duration altered the management in more than 2/3. Unknown upper tract involvement was diagnosed in 46% of patients and almost 40% of patients presented moderated to severe disease at VCE. In this group, therapy was changed in 67% of patients, with a significant increase in the use of immunosuppressors and biologicals, based solely on VCE findings. As it is well known that a suboptimal treatment of CD may predispose to a worse outcome, we strongly believe that evidence of mucosal healing should be thoroughly looked for in every affected bowel segments, and therapy escalated if lesions are detected, either by colonoscopy, VCE or radiological methods.

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Conclusions and Future Research

Referring to the described aims of this Thesis, the following conclusions can be formulated:

1. Both EBV and CMV were more prevalent in IBD patients than in healthy controls (HC). Both agents were more prevalent in areas of mucosa with endoscopic activity but there was no difference between CD and UC. Nevertheless, EBV median viral load was higher in inflamed mucosa of CD patients, while CMV median viral load was higher in ulcerated mucosa of UC patients. With respect to HHV6, the prevalence was similar in IBD patients and in HC. We also did not find any difference in viral serum prevalence between different treatment regimens challenging the idea that the level of immunosuppression per se would justify some observations of increased viral load in immunocompromised patients. Our findings suggest a potential role of virus in the immune disturbance present on IBD patient´s intestinal mucosa, with implication in disease pathogenesis, rather than disease severity.

2. We have demonstrated that both FC and FL are sensitive enough to monitor CD recurrence postoperatively, with a high negative predictive value. We have proposed a cut-off of 100 ug/g for FC and 7.25 ug/g for FL for predicting recurrence. We also compared the Modified Rutgeerts score and the Rutgeerts Score in predicting recurrent disease, and our results suggest that the Modified Rutgeerts score should be used in clinical practice, allowing to overcome some limitations of the Rutgeerts score. We propose serial monitoring of fecal markers in the context of postoperative Crohn´s disease, as an indicator of which and when, patients should be submitted to endoscopic evaluation.

3. In the context of asymptomatic anastomotic strictures, fecal markers performed equally well in predicting recurrence and being a noninvasive marker in selecting patients to EBD. In patients with high levels of fecal markers, as recurrent disease is highly probable, EBD should be performed, irrespective of symptoms, in order to confirm recurrence proximal to the anastomosis and escalate therapy if indicated. In this group, the best calculated cut-off value for FC was 90 ug/g and 5.6 ug/g for FL.

4. In our group of patients submitted to ileocolectomy, almost a third presented with anastomotic strictures. The performance of EBD was highly successful and safe with a technical success rate of 98%, a long-term efficacy of 37% and no major adverse events noted. Although repeating dilation was needed in 2/3 of patients, re-dilation was as technically successful, supporting the idea that this procedure can be done as needed. EDB demonstrated to be an effective and safe alternative to surgery, with a good short and long-term outcome, postponing and avoiding another surgery. EDB also allowed the diagnosis of disease recurrence in patients with no signs or biomarkers of active disease.

5. We found a good correlation between ileocolonoscopy, CTE and fecal markers in evaluating disease activity at diagnosis of CD and one year after immunosuppressive/biological therapy. In patients with endoscopic remission at one year, CTE findings significantly improved and the median CTE score significantly decreased. Wall hyperenhancement, mesenteric fat densification and comb sign significantly correlated with endoscopic disease activity and FC. A FC cut-off <100ug/g and a CTE score ≤ 3 points were found to be predictors of endoscopic remission. Both noninvasive markers may be used to monitor response to therapy.

6. Although data is scarse, our experience confirms that even in patients with long-time quiescent clinical disease, mucosal lesions may exist and VCE may be the only method to diagnose it. The recognition of active disease impacts patient management with therapy modifications.

Regarding the future and following the ideias that came up with this work, we believe it is important to rapidly standardize the methodology for fecal markers determination in order to establish definitive cut-off values for each clinical context. Non-invasive methods should be increasingly used to monitor disease activity, and selecting patients to endoscopic evaluation (colonoscopy and VCE). Endoscopy will retain its irreplaceable value as it allows direct visualization and sample collection. The modified Rutgeerts score should be validated in order to be widely used in clinical practice. The importance of transmural healing should be further evaluated in order to prevent bowel damage. A CTE score should be developed and disseminated in clinical practice.

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